

FY 1999-2001 Terminal Report on NASA Life Science Experiments.

Project Title: Greenhouse (III): Gas-Exchange and Seed-to-Seed Experiments on the Russian Space Station MIR and Earth-grown, Ethylene-treated Wheat Plants.

PI Name: William F. Campbell

PI Degree(s): Ph.D.

PI Address: Department of Plants, Soils and Biometeorology
Agricultural Sciences Building R109, MC 4820
Utah State University
4820 Logan Boulevard
Logan, UT 84322-4820

PI Phone: (435) 797-2253

PI Fax: (435) 797-3376

PI e-mail: bcampbell@mendel.usu.edu

Project Web Address:

Monitoring Center: ARC

Grant Number: NCC 2-831

NASA Solicitation: AO-OSSA-84

FY99 Funding: \$100,000

Start Date: 1993

End Date: 2001

Joint Agency: Dept. PSB/SDL, USU

Flight Assignment: NASA-Mir-1B

Co-Investigators

Affiliation:

- | | | |
|----|-----------------------|--|
| 1. | Bingham, Gail | Utah State University |
| 2. | Carman, John | Utah State University |
| 3. | Bubenheim, David | NASA Ames Research Center |
| 4. | Levinskikh, Margarita | Institute of Biomedical Problems, Russia |
| 5. | Sytchev, Vladimir N. | Institute of Biomedical Problems, Russia |
| 6. | Podolsky, Igor B. | Institute of Biomedical Problems, Russia |
| 7. | Chernova, Lola | Institute of Biomedical Problems, Russia |
| 8. | Nefodova, Yelena | Institute of Biomedical Problems, Russia |

NASA Facilities/Equipment used for this project:

1. Meetings to update research team and exchange ideas were held at Ames Research Center, Moffett Field, CA and Johnson Space Center, Houston, TX.
2. Laboratories and conference rooms at Kennedy Space Center were utilized when Shuttles returned from space with our plant samples.

Number of Funded Students:

Pre-college: (0) **Undergraduate:** (19) **Graduate:** (6) **Post-doctoral:** (1)

Sixteen of these 19 undergraduate students applied and were accepted into graduate school at various universities. These students were highly recruited by several universities such as North Carolina State, Iowa State, Illinois, Michigan State, Oregon State, Penn State, Utah State etc. Some of these students will soon graduate with M. S. degrees; others are aiming for higher degrees. All of the students indicated that they were influenced by their research experience on NASA projects.

Was a final report for this project submitted to NASA during FY99?

Yes, except for the current document 1999, to which we responded, we have not receive any documentation calling for a summary of activities for FY 2000-2001.

Abstract:

The Mir Space Station provided an outstanding opportunity to study long-term plant responses when exposed to a microgravity environment. Furthermore, if plants can be grown to maturity in a microgravity environment, they might be used in future bioregenerative life-support systems (BLSS). The primary objective of the Greenhouse experiment onboard Mir was to grow Super Dwarf and Apogee wheat through complete life cycles in microgravity; i.e., from seed-to-seed-to-seed. Additional objectives were to study chemical, biochemical, and structural changes in plant tissues as well as photosynthesis, respiration, and transpiration (evaporation of water from plants). Another major objective was to evaluate the suitability of the facilities on Mir for advanced research with plants. The Greenhouse experiment was conducted in the Russian/Bulgarian-developed plant growth chamber, the Svet, to which the United States added instrumentation systems to monitor changes in CO₂ and water vapor caused by the plants (with four infra-red gas analyzers monitoring air entering and leaving two small plastic chambers). In addition, the U.S. instrumentation also monitored O₂; air, leaf (IR), cabin pressure; photon flux; and substrate temperature and substrate moisture (16 probes in the root module). Facility modifications were first performed during the summer of 1995 during Mir 19, which began after STS-72 left Mir. Plant development was monitored by daily observations and some photographs. Plant samples

were collected five times during the 1995 experiment for chemical fixation or drying, and at final harvest. Samples were returned to Earth on STS-74 in November 1995. Because four of six light sets failed at the beginning of the experiment, plants grew very poorly; no seed heads were formed. The experiment was repeated in 1996 in the Svet greenhouse onboard the Russian Space Station, Mir, and in ground-based “mock-up” greenhouse controls as part of NASA 3, using new lamp banks and other equipment. Of the 104 seeds planted in the root module, 73 % of the seeds germinated in root compartment one and 56 % in two. The plants grew extremely well, producing far more biomass than in any other plant experiment in space and formed floral spikes, but seeds failed to form in the *ca.* 280 heads that developed. Plants were grown throughout the whole cycle of ontogenesis (123 d) with samples gathered to validate the morphological, embryological, cytological, reproductive and productive characteristics of the plants. We estimate that the fresh biomass would have exceeded a kilogram if all plants had been allowed to develop to maturity.

Plant samples were returned to Earth on STS-81 in January 1997. Comparative analyses showed that the number of tillers and flowers per spikelet were 63.2 % and 40 % greater, respectively, in flight-grown plants than in the ground-based controls. By contrast, the culm length (52.4 %), spike mass (49.2 %) and length (23.1 %), arista (awn) length (75.7 %) and number of spikelets per spike (42.8 %) from flight-grown plants were significantly less than the controls. Although wheat spikes were produced over a four to five week period, all florets ceased development at the onset of anthesis, resulting in 100 % sterility. Examination of the florets via light and scanning electron microscopy indicated that the anthers failed to dehisce and the pollen grains were smaller and shriveled compared to the ground-based controls, suggesting a chronic stress had occurred in the Svet growth chamber. At present, it appears that the failure of seed formation was caused by ethylene, a gaseous plant hormone, which was measured at 1.1 to 1.7 mg kg⁻¹ in the Mir cabin atmosphere, while CO₂ levels varied from 4,000 to 10,000 umol · mol⁻¹. Green plants from a second planting were harvested when they were 42 days old and frozen in the GN2 freezer.

Task Progress: (Please describe research accomplishments during the past year)

Iodine and silver fluoride were used to purify water onboard U. S. Shuttles and the Russian Space Station, Mir, respectively. In 1995, iodine-treated water, which ranged from 1.0 to 4.0 mg · kg⁻¹ with a mean of 2.9 mg · kg⁻¹ was applied to Super Dwarf wheat (*Triticum aestivum* L.) plants when Mir water (grey or tech grade) became scarce. Since the potential phytotoxicity of iodine and silver fluoride on Super Dwarf wheat is unknown and not a part of the experiments onboard Mir, we sought to determine whether these chemicals accounted for the subsequent poor wheat seedling growth and floral development onboard the Mir. Seedlings exposed to 1.0, 2.0 and 4.0 mg · kg⁻¹ of iodine or silver fluoride levels were not significantly different from the control shoot growth (Fig. 1). Although not shown, the root growth also showed a response similar to the shoot growth. Both disinfectants at 8 and 16 mg · kg⁻¹ showed significantly ($p \leq 0.01$) reduced seedling shoot and root lengths and fresh biomasses compared to the control and lower disinfectant levels (Tables 1 and 2).

Plant breeders have used 4,000 to 8,000 mg kg⁻¹ of the chemical herbicide, Ethereal, which degrades to ethylene gas, to induce male sterility in an attempt to produce hybrid cereals. However, a perusal of the literature indicates no controlled studies have been done to implicate ethylene at the levels measured on the Mir. To ascertain if Super Dwarf wheat plants' were sensitive to these levels of ethylene and whether failure of seed formation resulted, ground-based experiments were conducted to explore plausible conditions that would induce similar responses in plants observed on the Mir. Super Dwarf seeds were planted in 0.071 m² hydroponic plastic flats and covered with 2-mm diameter extruded diatomaceous earth and placed in six plexiglass hydroponic cylinders. The cylinders were then placed in a controlled-environment chamber. Pure CO₂ and ethylene gases were mixed with filtered air and wheat plants' exposure to ethylene gas dilutions of 0, 1, 3, 10 and 20 mg kg⁻¹ plus 1200 μ mol mol⁻¹ CO₂ began 7 d after emergence and continued through to maturity (Tables 3 and 4). Recirculating nutrient solutions specifically developed for wheat were pumped into each cylinder. Gas levels of each plant cylinder were controlled by small rotameters and monitored by gas chromatography. Plant density was thinned to approximately 30 plants per cylinder at emergence. Fourteen days after planting, the daily photoperiod was set to a 20/4 h d/n regime.

All ethylene-treated plants produced spikes at approximately the same time. However, plant height, awn length and the flag leaf were significantly shorter in the ethylene-exposed plants compared to the controls. Leaf epinasty (longitudinal leaf rolling) was visually evident at all ethylene levels and along with the above plant parameters appear to be sensitivity indicators of ethylene's presence in closed environments. Seed development and yield were normal in the control plants, but all ethylene-treated plants in this ground-based experiment exhibited 100 % sterility. In a later experiment, ethylene treatments of 0.0, 0.25, 0.50, 0.75 and 1 mg kg⁻¹ applied to the wheat cultivar, Apogee, significantly reduced seed yields compared to the controls (Fig. 2A & 2B). Also, there was a linear decrease in the above cited parameters with increasing ethylene. Interestingly, 1 mg kg⁻¹ ethylene was applied to one group of plants until the onset of anthesis and then removed through maturity (Fig. 3). Although these plants exhibited the sensitivity indicators of ethylene, removing the ethylene prior to anthesis showed no significant difference in seed yield compared to the controls.

Scanning electron microscopic examination of florets from Mir-grown and ethylene-treated Earth-grown plants showed that development ceased prior to anthesis and the anthers did not dehisce (see Figs 1-6 in attached manuscript). Laser scanning confocal microscopic examination of pollen grains from the Mir and ethylene-treated plants in the Earth experiment exhibited zero, one and occasionally two, but rarely three nuclei, whereas control plants were normally trinucleate. (see Fig 7 in attached manuscript). Of all the ground-based experiments we have conducted, which have included iodine, irradiance, temperature, CO₂ and ethylene levels, photoperiod and light intensity, only wheat plants exposed to ethylene gas have repeatedly exhibited responses that mimic those observed in plants grown on the Mir. The failure of anthers to dehisce, the paucity of trinucleate pollen and abrupt cessation of development of florets at the same stage of ontogeny in all plants suggested a chronic stress on the plants that induced male sterility. Since the literature is replete with the use of ethylene to induce male sterility in cereals, a strong case can be made that

the 1.1 to 1.7 mg kg⁻¹ of ethylene measured onboard the Mir Space Station could have accounted for the observed wheat sterility. Results of these studies provide essential prerequisites to the design and capacity of scrubbing equipment for hydrocarbons in BLSS. Resolving this limitation of space grown plants to provide food and other amenities to flight crews should be of high priority for future long-term space travels.

Earth Benefits: (Please update as necessary)

Plant physiologists have studied plant responses to gravity for well over a century, but we still have little understanding of how a plant can respond to even slight changes in the direction of gravitational acceleration. Tip a vertical stem of a seedling a few degrees from the vertical, and it will be vertical again within a few hours. Thus it would not be surprising if plants grew abnormally in a microgravity environment. Our Mir-grown experiment suggests that healthy plants can be grown in microgravity, and even that orientation will not be a serious problem. It is highly likely that suitable atmospheric control (e.g., elimination of ethylene) will permit seed formation and development in microgravity. Thus, there is little reason to doubt that wheat and other plants can be used as a food source for future astronauts, purifying the atmosphere in the process. The basic understanding gained in our space experiments, plus the ground studies that support the space experiment, may well have future application in agriculture as well as basic biology.

Bibliography Articles in Peer Reviewed Journals: (author(s), title, journal name, date)

1. Bingham, G. E., F. B. Salisbury, W. F. Campbell and J. G. Carman. **1994**. The Spacelab-MIR-1 "Greenhouse-2" experiment. *Microgravity Science and Technology* 18(3):58-65.
2. Salisbury, F. B., G. E. Bingham, W. F. Campbell, J. G. Carman, D. L. Bubenheim, B. Yendler and G. Jahns. **1995**. Growing Super-Dwarf wheat in *Svet* on *Mir*. *Life Support and Biosphere Science* 2:31-39.
3. Bingham, G. E., F. B. Salisbury, W. F. Campbell, J. G. Carman, B. Y. Yendler, V. S. Sytchev, Y. B. Berkovich, M. A. Levinskikh, and I. Podolsky. **1996**. The Spacelab-MIR-1 "Greenhouse-2" Experiment. *Microgravity Science and Technology*, 18 (5/4) p 225-232.
4. Carman, J. G., D. L. Bishop, and J. R. Hess. **1996**. Carbohydrates, minerals and free amino acids in *Triticum aestivum* L. kernels during early embryony. *J. Plant Physiol.* 149:714-720.
5. Banowetz, G. M., J. R. Hess, and J. G. Carman. **1997**. Monoclonal antibodies against the plant cytokinin, dihydrozeatin riboside. *Hybridoma* 16:479-483.

6. Bingham, G. E., D. K. Zhou, B. Y. Bartschi, G. P. Anderson, D. R. Smith, J. H. Chetwynd and R. M. Nadile. **1997**. CIRRIS-1A Calibration, Earth limb spectral measurements, and Atmospheric O₃, HNO₃, CFC-12, CFC-11 Profile Retrieval. *J. Geophysical Res.* Vol. 102, No. D3, p 3547-3558.
7. Rasmussen, R. D., D. Hole, J. R. Hess, and J. G. Carman. **1997**. Wheat kernel dormancy and abscisic acid levels following exposure to fluridone. *J. Plant Physiol.* 150:440-445.
8. Salisbury, F. B. **1997**. Growing Super-Dwarf wheat in Space Station Mir. *Life Support & Biosphere Science* 4:155-166.
9. Jiang, L., F. B. Salisbury, W. F. Campbell, J. G. Carman and R. Nan. **1998**. Studies on flower initiation of Super-Dwarf wheat (*Triticum aestivum* L.) under stress conditions simulating those on the space station Mir. *J. Pl. Physiol.* 152:323-327.
10. Salisbury, F. B., L. S. Gillespie, W. F. Campbell and P. Hole. **1998**. Ground-based studies with super-dwarf wheat in preparation for space flight. *J. Pl. Physiol.* 152:315-322.
11. Levinskikh, M. A., V. N. Sychev, T. A. Derendyaeva, O. B. Signalova, F. B. Salisbury, W. F. Campbell, D. Bubenheim. **1999**. Effects of the some space flight factors on growth and development of Super Dwarf wheat cultivated in greenhouse Svet. *Aviakosmicheskaya i Ekologicheskaya Meditsina* (Russia). Vol.33(2):37-41.
12. Veselova, T. D., G. M. Ilyina, H. H. Dzhalilova, M.A. Levinskikh, V. N. Sychev, F. B. Salisbury, W. F. Campbell. **1999**. The cytoembryologic investigation of Super Dwarf wheat grown on board the Mir orbital complex. *Aviakosmicheskaya i Ekologicheskaya Meditsina* (Russia), Vol.33(2):30-37.
13. Levinskikh, M. A., V. N. Sytchev, T. A. Derendyaeva, O. B. Signalova, F. B. Salisbury, W. F. Campbell, G. E. Bingham, D. L. Bubenheim and G. Jahns. **2000**. Analysis of the spaceflight effects on growth and development of Super Dwarf wheat grown on the Space Station Mir *J. Pl. Physiol.* 156:522-529.
14. Campbell, W. F., B. Bugbee, S. Klassen, E. Naegle, D. T. Strickland, F. B. Salisbury, G. E. Bingham, M. Levinskikh, G. Iljina, T. Veselova, V. N. Sytchev, I. Podolsky, W. R. McManus, D. L. Bubenheim, J. Stieber, and G. Jahns. **2001**. Comparative Floral Development of Mir- and Ethylene-treated Earth-grown Super Dwarf Wheat. *J. Pl. Physiol.* 158:1051-1060.
15. Monje, O., G. E. Bingham, F. B. Salisbury, W. F. Campbell, J. G. Carman, B. K. Eames, V. Sytchev, M. A. Levinskikh, and I. Podolsky. **2001**. Canopy photosynthesis and transpiration in microgravity: Gas exchange measurements aboard Mir. *Adv. In Space Res.* In Press.

16. Nan, R., J. G. Carman, F. B. Salisbury. **2001**. Effects of water stress and water logging on abscisic acid, cytokinins, and indole-3-acetic acid in 'super-dwarf' wheat (*Triticum aestivum* L.). J. Plant Physiol. In Press.
17. Salisbury, F. B., W. F. Campbell, J. G. Carman, G. E. Bingham, D. L. Bubenheim, B. Yendler, V. Sytchev, M. A. Levinskikh, I. Ivanova, L. Chernova and I. Podolsky. **2002**. Plant growth during the greenhouse II experiment on the MIR Orbital Station. J. Plant Physiol. In Press.

Articles in Other Journals (author(s), title, journal name, date)

NA

Abstracts (Papers-Presented at Regional and/or National Meetings):

1. Anderson, P., W. F. Campbell, F. B. Salisbury, J. L. Boettinger, T. Strickland, H. Biesinger, P. S. Hole, L. Gillespie, G. E. Bingham, M. Levinskikh, and I. Ivanova. **1996**. Clinoptilolite: A substrate for growing wheat in Svet aboard *Mir*. Western Society Crop Science, p. 9. Puyallap, Washington, June 24-26.
2. Biesinger, H., W. F. Campbell, T. Strickland, F. B. Salisbury, P. S. Hole, L. Gillespie, M. Levinskikh, and I. Ivanova. **1996**. Super Dwarf wheat responses to gradient irradiance. 77th Ann. Meet. ASHS-WR/PD-AAAS. Vol. 15:34. San Jose State University, June 23-27, 1996.
3. Strickland, D. T., H. Biesinger, W. F. Campbell, F. B. Salisbury, P. S. Hole, L. Gillespie, M. Levinskikh, and I. Ivanova. **1996**. Histochemical assessment of ground-based and *Mir*-grown wheat. 77th Ann. Meet. ASHS-WR/PD-AAAS. Vol. 15:55. San Jose State University, June 23-27, 1996.
4. Biesinger, H., D. T. Strickland, W. F. Campbell, F. B. Salisbury, and W. R. McManus. **1997**. SEM and EDX of crystals found on leaves of wheat grown under microgravity conditions. PD-AAAS/WR-ASHS Ann. Meet. June 22-26, 1997, Oregon State University, Corvallis, OR.
5. Strickland, D. T., W. F. Campbell, H. Biesinger, F. B. Salisbury and W. R. McManus. **1997**. SEM Investigations of reproductive morphology of *Mir*-grown wheat. PD-AAAS/WR-ASHS Ann. Meet. June 22-26, 1997, Oregon State University, Corvallis, OR.
6. Campbell, W. F. D. L. Bubenheim, F. B. Salisbury, B. G. Bugbee, G. E. Bingham, G. Iljina, T. Veselova, D. T. Strickland, W. R. McManus, M. Levinskikh, V. N. Sytchev, I. Podolski. **1998**. Ethylene-induced male sterility in wheat grown on *Mir*. Agron. Abstracts, p. 122

7. Naegle, E., D. Cook, D. T. Strickland, W. R. McManus, W. F. Campbell and B. Bugbee. **1998**. Morphometric and microscopic assessment of ethylene-treated wheat. 79th Ann. Meet. PD-AAAS/Biology 17(1):37. Ann. Meet. June 28-July 2, 1998, Utah State University, Logan, UT.
8. Klassen, S. P., W. F. Campbell and B. G. Bugbee. **1999**. Ethylene research at Utah State University. NASA-JSC PI Meeting, Houston, Texas, March 22-26, 1999.
9. Campbell, W. F., B. G. Bugbee, S. P. Klassen, D. T. Strickland, W. R. McManus, F. B. Salisbury, G. E. Bingham. **1999**. Ultrastructural characteristics of ethylene-induced sterile wheat florets. Agron. Abstract.
10. Strickland, D. T., W. F. Campbell, B. G. Bugbee, E. Naegle, S. P. Klassen, W. R. McManus, F. B. Salisbury and G. E. Bingham. **1999**. Assessment of reproductive failure of wheat grown on *Mir*. Plant Biology 99.

Abstracts – Proceedings [author(s), title, proceedings name and location, date]:

1. Bingham, G. E., F. B. Salisbury, L. S. Gillespie and W. Goncalves. **1994**. Diagnostic equipment for the "Greenhouse 2" experiment on the Russian Space Station, *Mir*. American Society for Gravitational Space Biology (ASGSB) Annual Meeting, San Francisco, CA. Oct. 20-22. Bulletin 8(1):84.
2. Salisbury, F. B., G. E. Bingham, L. S. Gillespie, J. G. Carman, W. F. Campbell, and D. L. Bubenheim. **1994**. Preparations for the "Greenhouse 2" experiment on the Russian Space Station, *MIR*. ASGSB Annual Meeting, San Francisco, CA. Oct. 20-22. Bulletin 8(1):70.
3. Anderson, P., W. F. Campbell, F. B. Salisbury, P. S. Hole, L. Gillespie and M. Levinskikh. **1995**. Overcoming post-harvest dormancy in Super Dwarf wheat. ASGSB Annual Meeting, Washington, D. C. Oct. 23-26. Bulletin 9(1):
4. Bingham, G. E., S. B. Brown and R. M. Mikesell. **1995**. GEMS measurements of plant response variables during Mir 19-20. Biomedical Space Research - Varna 95, Bulgarian Academy of Sciences, Varna, Bulgaria, Sept. 13-18.
5. Bingham, G. E., S. B. Brown and R. M. Mikesell. **1995**. A gas exchange measurement systems for plant growth experiments on the Mir Orbital Station. ASGSB Annual Meeting, Washington, D. C., Oct. 23-26. Bulletin 9(1):
6. Campbell, W. F., L. Avery, A. Landgraf, P. S. Hole, F. B. Salisbury, P. Anderson, L. Gillespie, G. Jahns and M. Levinskikh. **1995**. pH-induced discoloration of aldehyde fixatives during laboratory storage. ASGSB Annual Meeting, Washington, D. C., Oct. 23-26. Bulletin 9(1):

7. Hole, P. S., W. F. Campbell, F. B. Salisbury, P. Anderson, L. Gillespie and M. Levinskikh. **1995**. Stomatal density of Super-Dwarf wheat, *Triticum aestivum* L., as a function of temperature, light duration and CO₂ level. ASGSB Annual Meeting, Washington, D. C., Oct. 23-26. Bulletin 9(1):
8. Salisbury, F. B., G. E. Bingham, W. F. Campbell, J. G. Carman, P. Hole, L. S. Gillespie, V. N. Sytchev, Yu. Berkovitch, I. G. Podolski and M. Levinskikh. **1995**. Growing Super-Dwarf wheat through a life cycle on Space Station *Mir*. ASGSB Annual Meeting, Washington, D. C., Oct. 23-26. Bulletin 9(1):
9. Salisbury, F. B., G. E. Bingham, W. F. Campbell and J. G. Carman. **1995**. Super-Dwarf wheat growth response through a life cycle on Space Station *Mir*. Biomedical Space Research - Varna 95, Bulgarian Academy of Sciences, Varna, Bulgaria, Sept. 13-18.
10. Bingham, G. E., S. B. Brown, F. B. Salisbury, W. F. Campbell, J. G. Carman, G. Jahns, D. Pletcher, D. B. Bubenheim, B. Yendler, V. Sytchev, M. A. Levinskikh, I. Podolski, I. Ivanova, P. Kosgtov and S. Sapunovca. **1996**. Environmental measurements observed during the greenhouse-2 experiment on the *Mir* Orbital Station. 31st COSPAR Scientific Assembly, Birmingham, England, July 14-21. 31:364.
11. Bingham, G. E., S. B. Brown, F. B. Salisbury, W. F. Campbell, J. G. Carman, G. Jahns, D. Pletcher, D. B. Bubenheim, B. Yendler, V. Sytchev, M. A. Levinskikh, and I. Podolski. **1996**. Plant growth and plant environmental monitoring equipment on the *Mir* Space Station: Experience and data from the greenhouse II experiment. 31st COSPAR Scientific Assembly, Birmingham, England, July 14-21. 31:365.
12. Campbell, W. F., T. Strickland, D. Bubenheim, F. B. Salisbury, P. S. Hole, L. Gillespie, and M. Levinskikh. **1996**. Comparison of Long-Term Storage in Chemical Fixatives on Morphology and Anatomy of Super-Dwarf Wheat. 31st COSPAR Scientific Assembly, Birmingham, England, July 14-21. 31:365.
13. Campbell, W. F., P. Anderson, F. B. Salisbury, J. L. Boettinger, D. T. Strickland, H. Biesinger, P. S. Hole, L. Gillespie, G. E. Bingham, M. Levinskikh and I. Ivanova. **1996**. Effects of iodinated water on wheat in *Svet* aboard *Mir*. ASGSB Annual Meeting, Charlotte, N. C., Oct. 23-26. Bulletin 10(1):40.
14. Gillespie, L. S., F. B. Salisbury, W. F. Campbell and P. Hole. **1996**. Why were Super Dwarf wheat plants grown in space station *Mir* vegetative: Heat shock, short day, or microgravity? ASGSB Annual Meeting, Charlotte, N. C., Oct. 23-26. Bulletin 10(1):74.
15. Ivanova, T., P. Kostov, S. Sapunova, V. Sytchev, M. Levinskikh, I. Podolski, G. Bingham and F. Salisbury. **1996**. From Chinese cabbage to Super Dwarf wheat in the "Svet" greenhouse for future use in a Biological Life Support System. 47th IAF Congress, Beijing, China, Oct. 7-11.

16. Salisbury, F. B., G. E. Bingham, W. F. Campbell, J. G. Carman, P. Hole, L. S. Gillespie, V. N. Sytchev, I. B. Podolski, M. Levinskikh, D. L. Bubenheim and B. Yendler. **1996.** Experiments with Super-Dwarf wheat in space station *Mir*. ASGSB Annual Meeting, Charlotte, N. C., Oct. 23-26. Bulletin 10(1):34.
17. Salisbury, F. B., G. E. Bingham, W. F. Campbell, J. G. Carman, P. Hole, L. S. Gillespie, R. Nan, L. Jiang, V. N. Sytchev, I. Podolsky, M. Levinskikh, L. Chernova, I. Ivanova, D. L. Bubenheim and B. Yendler. **1996.** Growth of Super-Dwarf wheat on the Russian Space Station MIR. SAE Technical paper Series No. 961547, 26th Intl. Conf. Environ. Systems, Monterey, CA. July 8-11.
18. Bingham, G. E., S. B. Jones, D. Or, I. Podolski, M. A. Levinskikh, V. Sytchev, T. Ivanova, P. Kostov, S. Sapunova, I. Dandolov, D. B. Bubenheim and G. Jahns. **1997.** Microgravity effects on water supply and substrate properties in porous matrix root support systems. IAF/IAA-97-G.3.03, 48th International Astronautical Congress, Turin, Italy, Oct. 6-10.
19. Campbell, W. F., D. T. Strickland, F. B. Salisbury, and G. E. Bingham. **1997.** Ultrastructural assessment of reproductive structures of wheat grown on Mir. ASGSB Annual Meeting, Bulletin 11(1):40.
20. Ivanova, T., P. Kostov, S. Sapunova, I. Dandolov, V. N. Sytchev, I. Podolski, M. Levinskikh, G. Melesko, G. E. Bingham, F. B. Salisbury. **1997.** From fresh vegetables to the harvest of wheat plants grown in the Svet space greenhouse onboard the Mir Orbital Station. IAF Congress on Advanced Life Support Systems, Copenhagen, Denmark, April 25-26.
21. Podolski, I., V. N. Sytchev, M. A. Levinskikh, O. M. Strugov and G. E. Bingham. **1997.** Some peculiarities of water supply to the root medium during plant growth in microgravity. Second International Aerospace Congress. Moscow, Russia, Aug. 31-Sept. 5.
22. Strickland, D. T., W. F. Campbell, F. B. Salisbury, and G. E. Bingham. **1997.** Morphological assessment of reproductive structures of wheat grown on *Mir*. ASGSB Annual Meeting, Bulletin 11(1):14.
23. Campbell W. F., D. L. Bubenheim, B. G. Bugbee, F. B. Salisbury, G. E. Bingham, P. S. Hole, G. Iljina, T. Veselova, D. T. Strickland, E. Naegle, W. R. McManus, M. Levinskikh, V. N. Sytchev, I. Podolsky and G. Jahns. **1998.** Lessons learned from Greenhouse - Integrated wheat experiments in *Svet* on *Mir*. Third Phase 1 Research Symposium, Nov. 3-5, 1998, Huntsville, Alabama.

24. Monje, O., G. E. Bingham, J. G. Carman, W. F. Campbell, F. B. Salisbury, B. K. Eames, D. L. Bubenheim, V. Sytchev, M. A. Levinskikh, and I. Podolski. **1998**. Canopy photosynthesis and transpiration in microgravity: Gas exchange measurements aboard Mir. Symposium: F4.4 Flight Experiment Design and Flight Experiment Results in CELSS Research.
25. Klassen, S. P., W. F. Campbell and B. G. Bugbee. **1999**. Effects of low ethylene levels on crop plants. ICES, Denver, CO. July 12-15, 1999.
26. Levinskikh, M. A. , V. N. Sytchev, I. G. Podolsky, G. E. Bingham. **1999**. Final plant experiments on Mir provide second generation wheat and seeds. ASGSB Annual Meeting, 4-7 November, Seattle, WA.

Meeting Papers (Proceedings) (author(s), title, proceedings name and location, date)

1. Bingham, G. E., S. Jones, I. Podolsky (Moscow IBMP), and B. S. Yendler (NASA Ames, Moffett Field, CA), **1994**. Moisture Sensor for Use in Microgravity. Technical Report, NASA Ames, MIR Project Office, April 15.
2. Salisbury, F. B., W. F. Campbell, J. G. Carman and G. E. Bingham. **1995**. Growing Super-Dwarf wheat through a life cycle on Space Station *Mir*. Life Sciences & Space Medicine Conf., Houston, TX. April 3-7, 1995.
3. Yendler, B. S., G. Bingham, S. Jones, and I. Podolsky. **1995**. Moisture sensor for use in microgravity. SAE Tech. Paper No. 951471. 25th International Conference on Environmental Systems, 11-13 June, San Diego, CA.
4. Bingham, G. E., S. B. Jones, I. Podolski, and B. Yendler. **1996**, Porous Substrate Water relations observed during the greenhouse II flight experiment (MIR Space Station - 1995). 26th International Conference on Environmental Systems, Monterey, CA, July 8-11
5. Bingham, G. E., S. B. Brown, F. B. Salisbury, W. F. Campbell, J. G. Carman, G. Jahns, D. Pletcher, D. B. Bubenheim, B. Yendler, V. Sytchev, M. A. Levinskikh, and I. Podolski. **1996**, Plant growth and plant environmental monitoring equipment on the Mir Space Station: Experience and data from the Greenhouse II experiment. 26th International Conference on Environmental Systems, Monterey, CA, July 8-11
6. Salisbury, F. B, G. E. Bingham, W. F. Campbell, J. G. Carman, P. Hole, L. S. Gillespie, V. Sytchev, M. A. Levinskikh, I. Podolski, D. B. Bubenheim, and B. Yendler,. **1996**, Growth of Super Dwarf wheat on the Russian Space Station MIR. SAE Technical Paper Series No. 961392, 26th International Conference on Environmental Systems, Monterey, CA, July 8-11

7. Campbell, W. F., D. L. Bubenheim, F. B. Salisbury, G. E. Bingham, L. S. Gillespie, J. G. Carman, G. Fljina, V. Tatijana, D. T. Strickland, W. R. McManus, M. Levinskikh, V. N. Sytchev, I. Ivanova, L. Chernova and I. Podolski. **1997**. Morphometric plant growth and light and scanning electron microscopic assessment of reproductive structures of wheat grown on Mir. Phase 1 Research Program Results Symposium. NASA/JSC, Houston, Texas, August 5-7
8. Bingham, G. E., W. F. Campbell, F. B. Salisbury, J. G. Carman, G. Jahns, D. Pletcher, D. B. Bubenheim, B. Yendler, V. Sytchev, M. A. Levinskikh, I. Podolski, T. G. Ivanov, P. Kostov, and S. Supernova, **1997**. Integrated plant experiments on the Mir Space Station. Phase 1 Research Program. Interim Results Symposium, JSC, Houston, TX. August 5-7.
9. Campbell, W. F. **1997**. Morphometric and morphological characteristics of wheat grown onboard Mir. NASA-3, Mir-10-20 Science Working Group Conference, July 7-11, 1997, Moscow, Russia.
10. Salisbury, F. B., M. Levinskikh, G. Bingham, W. F. Campbell, J. G. Carman and D. Bubenheim. **1997**. Greenhouse - integrated plant experiments on Mir. Phase 1 Research Program Quarterly Research Report. Johnson Space Center, Houston, TX. pp. 3-17 to 3-58.
11. Bingham, G. E., W. F. Campbell, J. G. Carman, S. B. Jones, D. Or, O. Monje, V. N. Sytchev, M. A. Levinskikh, I. G. Podolski, T. Ivanova, P. Kostov, S. Sapunova, I. Dandolov, D. B. Bubenheim, and G. Jahns. **1998**. NASA-Mir greenhouse experiments with wheat. Second Phase 1 Research Program Results Symposium, NASA/ARC, Moffett Field, CA, March 31-April 1, 1998.
12. Campbell, W. F., D. L. Bubenheim, F. B. Salisbury, G. E. Bingham, G. Fljina, V. Tatijana, D. T. Strickland, W. R. McManus, M. Levinskikh, V. N. Sytchev, I. Ivanova, L. Chernova, and I. Podolski. **1998**. Male sterility in Mir-grown wheat. Second Phase 1 Research Program Results Symposium, NASA/ARC, Moffett Field, CA, March 31-April 1, 1998.
13. Campbell W. F., D. L. Bubenheim, B. G. Bugbee, F. B. Salisbury, G. E. Bingham, P. S. Hole, G. Iljina, T. Veselova, D. T. Strickland, E. Naegle, W. R. McManus, M. Levinskikh, V. N. Sytchev, I. Podolsky and G. Jahns. **1998**. Lessons Learned from Greenhouse - Integrated Wheat Experiments in *Svet* on *Mir*. Third Phase 1 Research Symposium, Nov. 3-5, 1998, Huntsville, Alabama.
14. Monje, O., B. Bugbee and G.E. Bingham. **1998**. Modeling Canopy Transpiration: Lessons form Controlled Environments. Crop Simulation Workshop. Beltsville, MD. April 5-8.

Academic Dissertations / Theses (author, title, institution, date)

1. Liming Jiang. 1997. Development of Super-Dwarf wheat under stress conditions simulating those on the Space Station, Mir. M. S. Thesis. Utah State University Library.
2. Ruben Nan. 1997. Effects of CO₂, light, photoperiod and water potential on levels of hormones in wheat (*Triticum aestivum* L.). Ph. D. Dissertation. Utah State University Library.

Books or Book Chapters (author(s), chapter title, book title, publisher, date)

NA

Government Publications (author(s), title, technical memorandum number, date)

NA

Presentations / Lectures (speaker, title, forum title and location, date)

NA

Patents (author(s), title, patent number or pending designation, date)

NA

Awards (awardee, title of award, presenting organization, date)

1. **Biesinger, H., W. F. Campbell, T. Strickland, F. B. Salisbury, P. S. Hole, L. Gillespie, M. Levinskikh, and I. Ivanova. 1996. Super Dwarf Wheat Responses to Gradient Irradiance. 77th Ann. Meet. Western Region-American Society for Horticultural Science -- Pacific Division of the American Association for the Advancement of Science (WR-ASHS/PD-AAAS). Vol. 15:34. San Jose State University, June 23-27, 1996. (Award For Excellence in Student Poster Paper Competition).**

2. **Strickland, D. T., H. Biesinger, W. F. Campbell, F. B. Salisbury, P. S. Hole, L. Gillespie, M. Levinskikh, and I. Ivanova. 1996. Histochemical Assessment of Ground-based and Mir-grown Wheat. 77th Ann. Meet. WR-ASHS/PD-AAAS. Vol. 15:55. San Jose State University, June 23-27, 1996. (Award For Excellence in Student Poster Paper Competition).**
3. **Biesinger, H., D. T. Strickland, W. F. Campbell, F. B. Salisbury, and W. R. McManus 1997. SEM and EDX of Crystals Found on Leaves of Wheat Grown under Microgravity Conditions. PD-AAAS/WR-ASHS Ann. Meet. June 22-26, 1997, Oregon State University, Corvallis, OR. (Award For Excellence in The WR-ASHS Student Poster Paper Competition).**
4. **Strickland, D. T., W. F. Campbell, H. Biesinger, F. B. Salisbury and W. R. McManus. 1997. SEM Investigations of Reproductive Morphology of *Mir*-Grown Wheat. PD-AAAS/WR-ASHS Ann. Meet. June 22-26, 1997, Oregon State University, Corvallis, OR. (Award For Excellence in Student Poster Paper Competition in The WR-ASHS, The PD-AAAS-wide Robert I. Larus Travel Award, And An Honorable Mention at The National AAAS Meeting, Philadelphia, Pa, Feb. 1998).**
5. **Naegle, E., D. Cook, D. T. Strickland, W. R. McManus, W. F. Campbell and B. Bugbee. 1998. Morphometric and Microscopic Assessment of Ethylene-Treated Wheat. 79th Ann. Meet. PD-AAAS/Biology 17(1):37. Ann. Meet. June 28-July 2, 1998, Utah State University, Logan, UT. (Award for Excellence in Student Poster Paper Competition in the Biological Sciences Division and the PD-AAAS-wide Award for the Best Student Poster Paper).**
6. Group Achievement Award from The National Aeronautics and Space Administration (NASA) "In recognition of their outstanding contribution in the support, development, operations, and implementation of NASA's objectives in the Shuttle/MIR Science Program, 1997."
7. NASA/USU Research Team were Finalist in 1998 in the USU's Robins Achievement Awards – "Growing Wheat in Space."

Other:

1. Campbell, W. F. 1994. Preparation for *MIR* Space Station production. Science Workshop June 18-23, 1994, NASA/ARC, Moffett field, CA.
2. Campbell, W. F. 1994. Germination, growth and development of wheat in preparation for *MIR* space station production. Science Workshop, Nov. 14-18, 1994. Institute of Biomedical Problems, Moscow, Russia.

3. Campbell, W. F. **1996**. Growing Wheat in Space. Great Salt Lake Chapter Health Physics Society. June 29, 1996. USU Campus.
4. Levinskikh, M. A., V. N. Sytchev, I. G. Podolski, T. A. Deredjaeva, F. B. Salisbury and W. F. Campbell. **1997**. Peculiarities of Super Dwarf wheat growth and development in greenhouse *Svet* in ground and space experiments. NASA-3, Mir-10-20 Science Working Group Conference, July 7-11, 1997, Moscow, Russia.

Impact on America (This information is for internal NASA use only; it will not be published).

Information and experience we have gained in attempting to grow wheat plants from seed-to-seed on the Russian Space Station, Mir, will lead to the successful growing of plants in microgravity environments.

Who Uses This Research (Briefly describe what other researchers are using these results)

Various disciplines involved with constructing Svet growth chambers or greenhouses to be used on the International Space Station. Also, industries involved in developing instruments to monitor the responses of plants to environmental changes occurring at different stages of ontogeny.

Industrial Affiliates (Please list the names of industrial affiliates)

NA

New Technologies (What new technologies have emerged from this research)

A new and improved instrumentation system was developed to monitor changes in CO₂ and water vapor caused by the plants (with four infra-red gas analyzers monitoring air entering and leaving two small plastic chambers) in Svet onboard the Mir. In addition, the U. S./Russian team improved instrumentation to monitor O₂; air, leaf (IR), cabin pressure; photon flux; and substrate temperature and moisture (16 probes in the root module).

Research and development of a new wheat cultivar, Apogee, which has greater resistance to ethylene gas than does the Super Dwarf. In recent experiments, Apogee has developed seed while growing on the Mir, thus, indicating differential tolerance to ethylene gas.

Our recent experiments suggest that healthy plants can be grown in microgravity, and perhaps that even orientation will not be a serious problem. We have demonstrated that ethylene only affects the wheat plants during anthesis (probably during meiosis). It is highly likely that atmospheric ethylene can be scrubbed during this critical stage of ontogeny, thereby, permitting seed formation and development in microgravity. Thus, we are optimistic that Apogee wheat or newer cultivars and other plants can be used for food and oxygen production while eliminating carbon dioxide and nitrogenous wastes, basic aims of BLSS, which are critical to mankind's long-term presence in space or reduced gravity environments. Also, the basic information gained in our space and ground-based research may well have future application in agriculture as well as basic biology.

Current Employers of Former Graduate Students (Please list current employers)

1. Liming Jiang is working for a commercial greenhouse in Virginia.
2. Ruben Nan is working for DeKalb Seed Company, which is a subsidiary of Monsanto Chemical Co.

Magazine Covers (Publication, date, brief description)

The wheat pollen grown on the Russian Space Station, Mir, was magnified about 500 times and shown on a locally published magazine cover.

Popular Press Coverage (Please list coverage from newspapers, radio, television, etc)

Newspaper Items describing the growing of wheat in space were published in the Herald Journal, Logan and the Salt Lake Tribune, Salt Lake City, UT.

September 23, 1999

USU SCIENTISTS AWAIT FIRST SPACE WHEAT FROM SPACE-GROWN SEEDS

LOGAN – There were just four tiny, very important seeds, and plant scientists at Utah State University could't wait to get a first-hand look at them. The wheat seeds, which recently were returned to Earth with the last cosmonauts from the Russian Mir Space Station, are the only known living things (other than fungi) that were grown in space from seeds harvested from other space-grown plants.

Project manager Gail Bingham, Professor of plants, soils and biometeorology and researcher at the Space Dynamics Laboratory, said the USU team expects to get its first hands-on look at the seeds in late October when a colleague from the Institute of Biomedical Problems Research in Moscow arrives in Logan. Plant scientists Bill Campbell, John Carman and Bruce Bugbee plan to conduct germination and genetic experiments with the seeds at USU

The wheat variety, named Apogee, is a fast-growing, dwarf variety specially bred by Bugbee in a NASA-supported project. Apogee was developed for its compact size suited for use in small growth chambers typical of those used for space experiments.

USU's connection with space farming aboard Mir goes back to 1995 when an experiment produced a crop of strangely twisted plants. That first wheat crop was planted by U. S. Astronaut Shannon Lucid during her six month stay aboard Mir and tended and harvested by John Blaha who took her place on the space station crew, looked very promising. But when scientists on the ground got a look at the wheat they found the experiment had produced beautiful, but sterile plants with so seeds in the heads.

"Ground testing and studying data from the space growth chamber have confirmed that there was a build-up of ethylene gas in the Mir Cabin," Bingham said. "Ethylene is a plant hormone used in plant breeding to suppress yield. And we got sterile plants in ground control tests when plants were exposed to ethylene."

Bingham said after the U. S. left Mir the Utah State group continued to work with the Russians and Apogee was planted. Although they had very low germination rates – just 10 percent compared with 95 percent on the ground – 20 plants grew and produced the same seed numbers as did ground control plants. More than 500 of those seeds returned to Earth with cosmonauts in March 1999 and 10 seeds from the crop of space wheat were left aboard Mir and planted.

One of the ten seeds produced a plant with the usual two heads, Bingham said, and those two heads produced four seeds.

"We checked with ground controllers and found that they were concerned about methane build-up on Mir at the time the first generation of wheat was growing so they ran scrubbers for that which also scrubbed ethylene out of the air. That helped improve conditions for us too," Bingham said. The second generation of plants produced the kind of yield we see in the presence of ethylene."

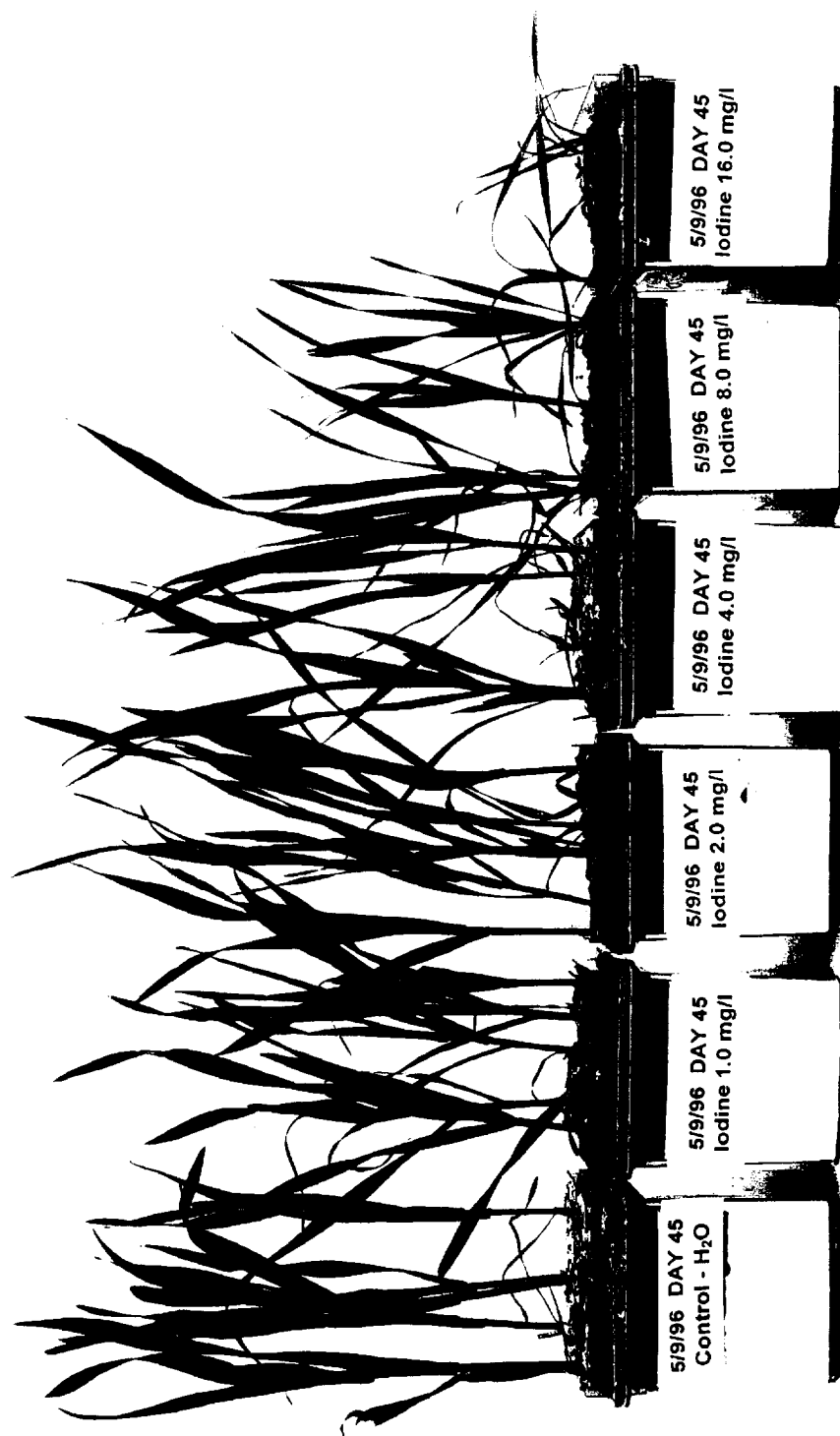
The USU team isn't just sitting back and waiting for the space seeds to arrive. They are busy conducting more ground-based tests in carefully controlled growth chambers and working with their Russian colleagues to propose other space-based experiments. While some talk of Mir still being in usable condition, Bingham

believes its days as a microgravity laboratory are over.

“The Russians are in such terrible financial shape I think they’re being forced to admit that they just can’t support Mir any longer,” Bingham said. “We are negotiating with the Institute for Biomedical Problems in Moscow for new experiments on the International Space Station.”

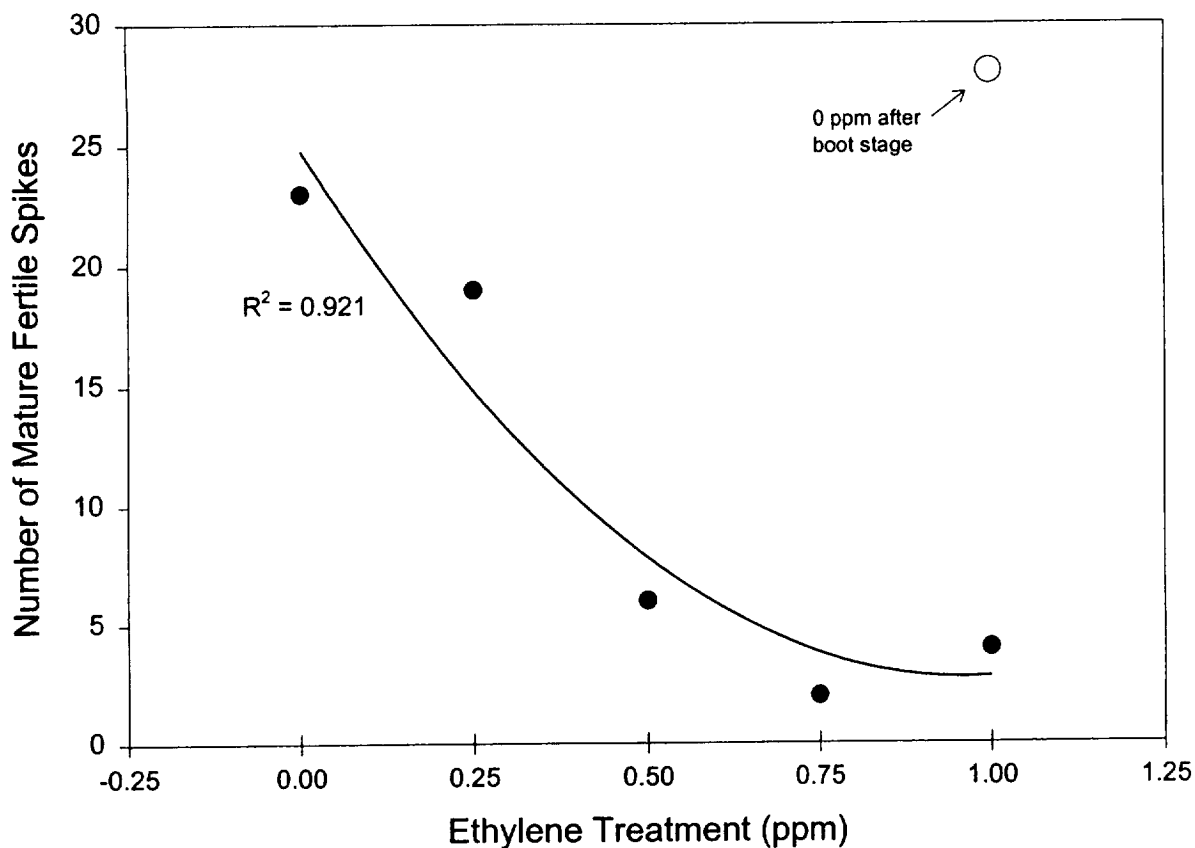
USU can offer hardware for the experiment and the science already done by Bingham, Bugbee and Campbell. The Russian scientists can offer expertise gained through years of using Mir as a microgravity laboratory and the earliest opportunity for USU to have long-duration experiments in space.

“There are about 400 people with proposals competing for the first four U. S. slots on the International Space Station,” Bingham explained. “Working with the Russians means we are one of just 2 or 3 proposals for their slots.



Number of Mature Fertile Wheat Spikes as a Function of Ethylene Treatment

Fig. 2A



Wheat Seed Production as a Function of Ethylene Treatment

Fig. 2B

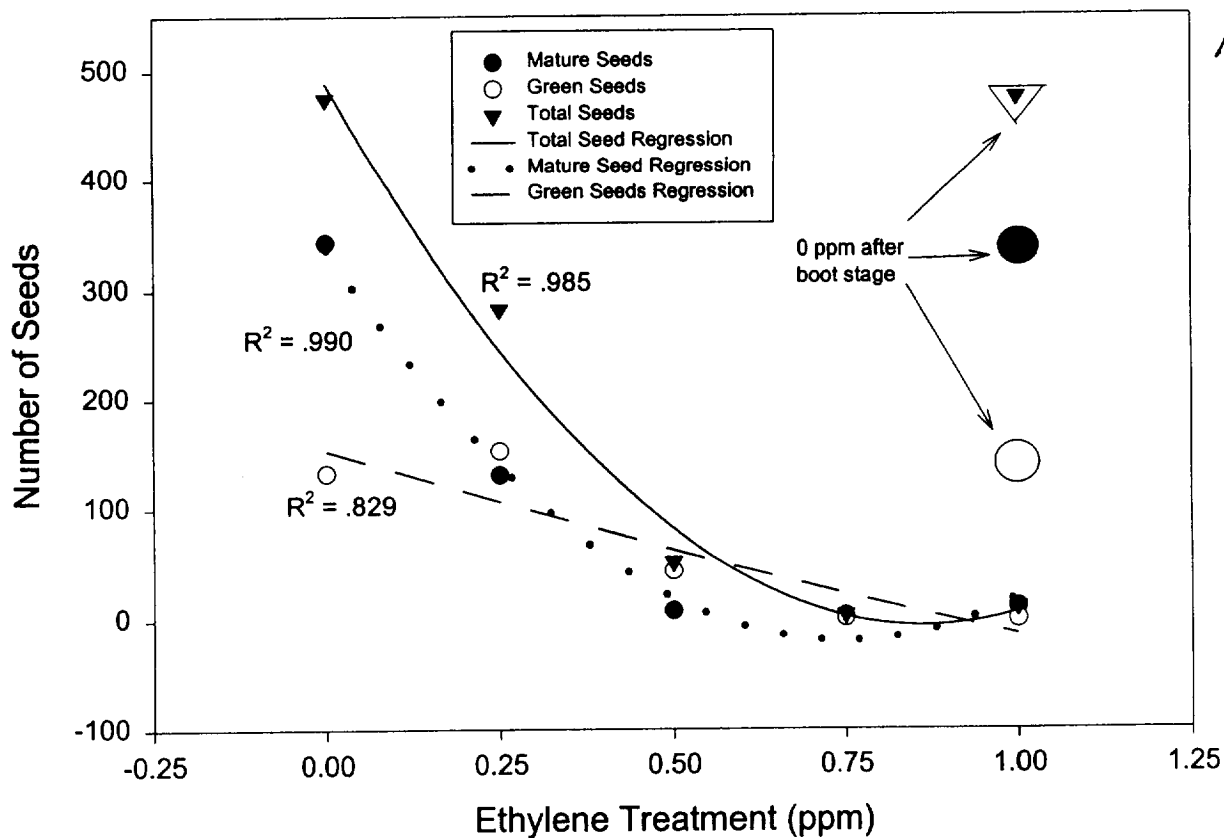


Fig. 3

Average Number of Seeds per Spike versus Ethylene Treatment

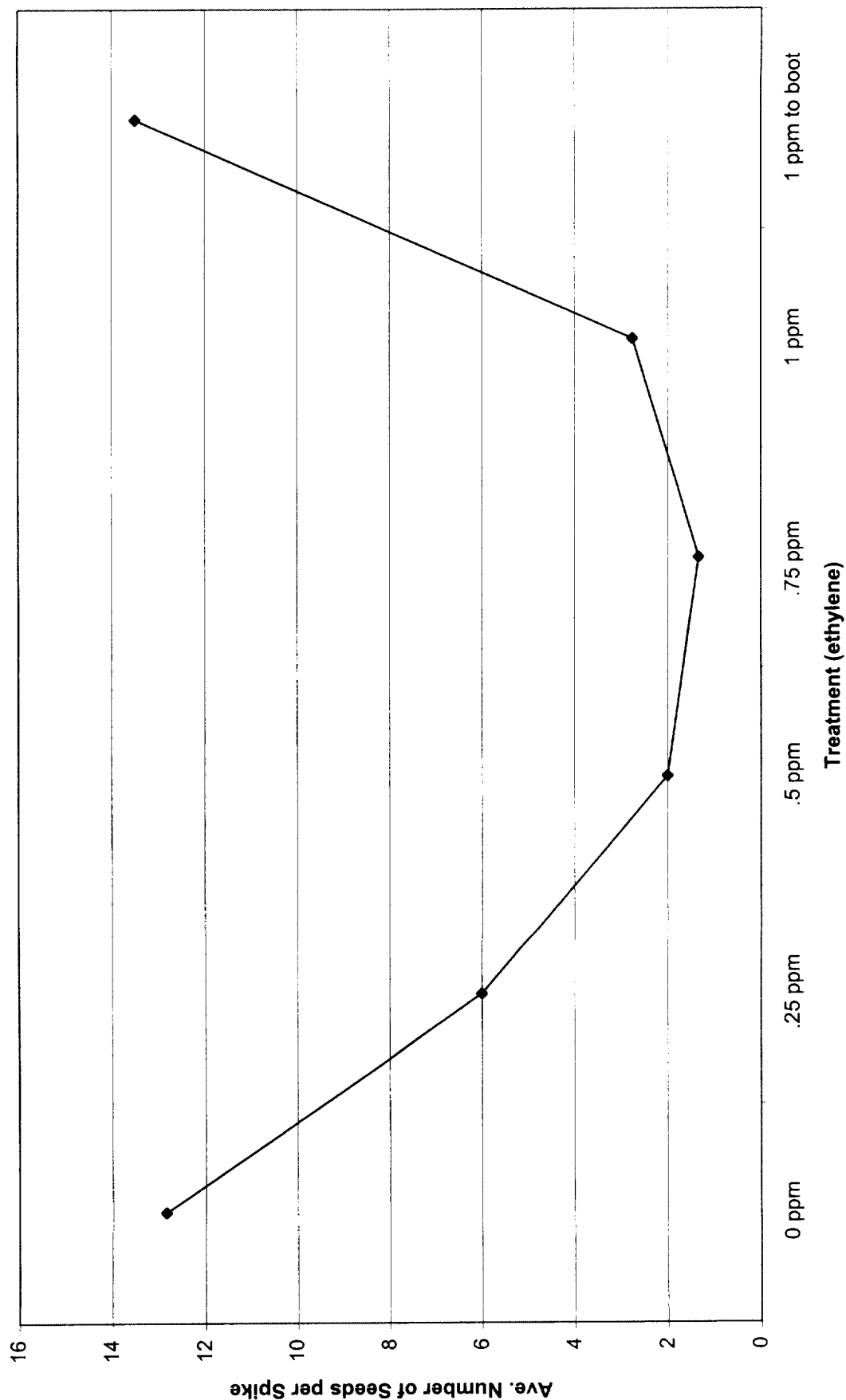


Table 1. Effects of Iodine- and Silver Fluoride-treated Water on Super Dwarf Wheat Seed Germination, Shoot and Root Length and Fresh Biomass (Paper Towels, 8 d).

Level (mg/kg)	Seed Germ. [@]	Length (cm)		Fresh Biomass (g)	
		Shoot	Root	Shoot	Root
----- AgF -----					
0.0	85 ab	11.4 e	9.5 a	3.1 c	3.7 b
1.0	85 ab	11.4 e	20.0 a	2.9 c	2.7 c
2.0	80 ab	11.2 ef	19.6 a	2.5 d	4.3 a
4.0	90 a	10.7 ef	15.1 b	2.2 d	3.4 c
8.0	83 ab	10.8 ef	14.7 bc	2.5 d	3.1 cd
16.0	78 b	9.7 g	14.2 c	2.3 d	3.0 de
----- Iodine -----					
0.0	83 ab	15.3 c	18.6 b	5.1 b	2.7 e
1.0	73 c	16.5 b	19.2 a	4.7 b	2.3 f
2.0	59 d	17.8 a	20.9 a	7.3 a	3.2 c
4.0	64 d	14.8 d	17.5 c	5.0 b	1.9 g
8.0	59 d	10.0 g	10.9 g	1.0 f	1.4 h
16.0	43 e	8.7 h	11.4 f	1.4 e	0.8 i

[@]Letters Following Numbers Within a Column Indicate they are Significantly Different $p \leq 0.01$.

Table 2. Effects of Iodine and Silver Fluoride-treated Water on the Number of Spikelets per Spike, Florets per Spikelet, Seeds per Spike and Seed Weight per Spike of Super Dwarf Wheat

Level (mg/kg)	Spikelets/ Spike	Florets/ Spikelet	Seeds/ Spike	Seed Wt (g)/ Spike
----- AgF -----				
0.0	20.3 c	3.0 c	14.6 b	2.6 b
1.0	20.8 b	3.1 c	10.3 d	2.5 b
2.0	22.6 a	2.5 d	14.5 b	2.4 b
4.0	21.3 b	3.1 c	15.3 b	3.2 a
8.0	9.7 g	2.5 d	5.8 f	1.2 d
16.0	9.5 g	2.4 d	5.4 g	1.5 d
----- Iodine -----				
0.0	22.0 a	3.1 c	17.5 a	3.1 a
1.0	21.0 b	4.0 a	15.2 b	2.3 b
2.0	19.1 d	3.0 c	13.8 c	2.5 b
4.0	18.3 e	3.5 b	13.8 c	1.6 c
8.0	15.2 f	2.8 cd	7.0 e	0.8 e
16.0	0.0 h	0.0 e	0.0 h	0.0 f

@Letters Following Numbers Within a Column Indicate they are Significantly Different $p \leq 0.01$.

Table 3. Morphometric Responses of Earth-grown Super Dwarf Wheat Plants at Elevated CO₂ (1200 mmol mol⁻¹) and Varying Levels of Ethylene Gas – Logan, UT.

Ethylene Level mg kg ⁻¹	Plant Height (mm) [®]	No. of Tillers	Spike Ln (mm)	Awn Ln (mm)	No. Rachis Nodes	Florets/ Spikelet	Percent Fertility
0	391 a	5.6 d	57.9 a	50.9 a	17.2 a	3.9 d	53.0 a
1	275 b	8.0 c	44.2 b	8.8 b	17.2 a	10.1 c	0.0 b
3	251 c	12.6 b	40.4 c	6.7 c	16.8 a	12.5 a	0.0 b
10	221 d	15.4 a	40.0 c	6.6 c	16.2 b	11.1 b	0.0 b
20	199 e	15.2 a	43.8 b	9.3 b	15.8 b	11.5 b	0.0 b

[®]Letters Following Numbers Within a Column Indicate They Are Significantly Different $P \leq 0.01$

Table 4. Morphometric Responses of Super Dwarf Wheat Tillers Grown at Elevated CO₂ and Varying Levels of Ethylene Gas.
Logan, UT

Ethylene Level mg kg ⁻¹	No. Leaves/ Tiller [@]	Spike Ln (mm)	Awn Ln (mm)	No. Rachis Nodes	Florets/ Spikelet	Percent Fertility
0	3.8 bc	50.9 a	45.0 a	17.8 a	3.5 d	32.0 a
1	4.2 a	47.5 b	14.5 b	13.3 b	11.7 a	1.7 b
3	3.8 bc	39.7 c	6.7 d	14.1 b	10.6 a	0.0 c
10	4.2 a	36.5 d	8.0 c	12.2 b	7.6 c	0.0 c
20	3.9 bc	37.2 d	8.9 c	12.6 b	8.1 b	0.0 c

[@]Letters Following Numbers Within a Column Indicate They Are Significantly Different $P \leq 0.01$.

Comparative floral development of Mir-grown and ethylene-treated, earth-grown Super Dwarf wheat

William F. Campbell¹, Frank B. Salisbury^{1*}, Bruce Bugbee¹, Steven Klassen¹, Erin Naegle¹, Darren T. Strickland¹, Gail E. Bingham¹, Margarita Levinskikh², Galena M. Iljina³, Tatjana D. Veselova³, Vladimir N. Sytchev², Igor Podolsky², William R. McManus⁴, David L. Bubenheim⁵, Joseph Stieber⁵, Gary Jahns⁵

¹ Plant, Soils & Biometeorology Department, Utah State Univ., Logan, UT 84322-4820, U.S.A.

² Institute of BioMedical Problems, 123007, Khoroshevskoe shosse 76 a, Moscow, Russia

³ Moscow State University, 119899, Biological Faculty, Vorobievsky Gory, Moscow, Russia

⁴ Biology Department Utah State Univ., Logan, UT 84322-5305, U.S.A.

⁵ NASA/Ames, Moffett Field, CA 94035, U.S.A.

Received November 27, 2000 · Accepted February 6, 2001

Summary

To study plant growth in microgravity, we grew Super Dwarf wheat (*Triticum aestivum* L.) in the Svet growth chamber onboard the orbiting Russian space station, Mir, and in identical ground control units at the Institute of BioMedical Problems in Moscow, Russia. Seedling emergence was 56 % and 73 % in the two root-module compartments on Mir and 75 % and 90 % on earth. Growth was vigorous (produced ca. 1 kg dry mass), and individual plants produced 5 to 8 tillers on Mir compared with 3 to 5 on earth-grown controls. Upon harvest in space and return to earth, however, all inflorescences of the flight-grown plants were sterile.

To ascertain if Super Dwarf wheat responded to the 1.1 to 1.7 $\mu\text{mol} \cdot \text{mol}^{-1}$ atmospheric levels of ethylene measured on the Mir prior to and during flowering, plants on earth were exposed to 0, 1, 3, 10, and 20 $\mu\text{mol} \cdot \text{mol}^{-1}$ of ethylene gas and 1200 $\mu\text{mol} \cdot \text{mol}^{-1}$ CO_2 from 7 d after emergence to maturity. As in our Mir wheat, plant height, awn length, and the flag leaf were significantly shorter in the ethylene-exposed plants than in controls; inflorescences also exhibited 100 % sterility. Scanning-electron-microscopic (SEM) examination of florets from Mir-grown and ethylene-treated, earth-grown plants showed that development ceased prior to anthesis, and the anthers did not dehisce. Laser scanning confocal microscopic (LSCM) examination of pollen grains from Mir and ethylene-treated plants on earth exhibited zero, one, and occasionally two, but rarely three nuclei; pollen produced in the absence of ethylene was always trinucleate, the normal condition. The scarcity of trinucleate pollen, abrupt cessation of floret development prior to anthesis, and excess tillering in wheat plants on Mir and in ethylene-containing atmospheres on earth build a strong case for the ethylene on Mir as the agent for the induced male sterility and other symptoms, rather than microgravity.

Key words: ethylene – microgravity – Mir – pollen – seed set – sterility – Super Dwarf – Svet – *Triticum aestivum* – wheat

* E-mail corresponding author: FrankSals@aol.com

Introduction

Plants are known to be extremely sensitive to gravity. A young plant stem that is growing vertically, for example, when placed on its side or tipped only a few degrees from the vertical, will right itself within a few hours (e.g., Salisbury 1993). Thus, it is of great interest to study plant growth in microgravity. If plants can be grown through a complete life cycle (i.e., from seed-to-seed) in microgravity, we can conclude that there is no physical, chemical, or biological process that is so dependent on gravity that plants cannot mature in its absence. It has long been a goal of researchers in the field of plant gravitropism, as well as space biologists, to see if, in the microgravity of an orbiting space vehicle, it is possible to grow plants that are comparable in every important way with plants grown on earth under identical environmental conditions except for gravity.

There is also a practical reason to grow highly productive food plants in space: Such crops might supply oxygen, food, and water and might remove CO₂ and nitrogenous wastes produced by human occupants of the space craft. Crops used this way would be part of a *bioregenerative life-support system* (BLSS). Such a BLSS could be critical to mankind's long-term presence in space or reduced gravity environments as on the Moon or Mars (Dutcher et al. 1994, Halstead and Dutcher 1987, Kuang et al. 1995, Salisbury 1999).

Wheat has been shown to produce extremely high yields in controlled environments – nearly six times the world record yields in the field (Bugbee and Salisbury 1988, 1989). During 1995 and 1996 in a joint United States/Russian project, we grew a short cultivar of wheat, called «Super Dwarf,» in the Russian space station Mir (Bingham et al. 1995, 1997, Salisbury 1997). Mechanical problems plagued us in 1995, but these were remedied, and we completed a 123-day experiment in 1996 as well as a 39-day experiment in 1996/97. The plants grew extremely well, producing more plant biomass than in any previous space experiment (thanks to higher irradiance than in previous experiments), and in the 123-day experiment, there were ca. 280 wheat heads produced. When the plants were returned to earth, however, all the heads proved to be sterile. Not a single seed was produced.

In our post-mortem discussions, we listed about a dozen reasons why seeds might not have formed. Principal among these were high CO₂ water-logged substrate, anoxia, ethylene, and microgravity. Because density-driven convection does not occur in microgravity, Kuang et al. (1993) suggested that a high boundary layer of humidity might have caused the failure of anthers to dehisce, or oxygen deprivation might have impacted the anthers. Indeed, Kuang et al. (1995) observed that after 6 d in spaceflight onboard the space shuttle <Endeavour> (STS-54), *A. thaliana* exhibited collapsed ovaries with empty ovules and deformed empty microspores. Ethylene was not present in the post flight analysis of gas in each chamber. Instead, Kuang et al. (1995) at-

tributed the problem to O₂ depletion in the boundary layer; they demonstrated a solution by stirring the air in the chamber during a later experiment, called Chromax 5. Murgia et al. (1992) have shown that pollen viability is severely decreased by anoxia. Also, Quebedeaux and Hardy (1973) observed that a 5% oxygen atmosphere completely inhibited seed development in soybean.

None of these suggestions applies to our experiment, however, because air was never stagnant in Mir's growth chamber (called Svet); fans pulled it over the plants and past the lamps. And with the lights on, those green plants were probably producing oxygen that kept their boundary layers well supplied.

Another possible cause of sterility is root-zone anoxia, which might have occurred for short periods during programmed water-status changes (Bingham et al. 1996 a, b, 1997, Yendler et al. 1996), mimicking the low atmospheric oxygen content observed in flooded soils on earth (Thomson and Greenway 1991). This seems unlikely, however, because root-zone anoxia, if it occurred, was sporadic, whereas wheat heads that formed over a six-week period were *all* sterile.

Wheat plants grown onboard Mir were exposed to high levels of CO₂ (4,000 to 10,000 μmol · mol⁻¹, continuously monitored by our infrared gas analyzer), which were super-optimal and shown to reduce yield in wheat (Grotenhuis and Bugbee 1997, Jiang et al. 1998, Salisbury 1997, Voeselek et al. 1997). Bugbee et al. (1994) and Grotenhuis and Bugbee (1997) observed that elevating CO₂ levels from 350 to 1,200 μmol · mol⁻¹ increased the seed yield of wheat and rice, *Oryza sativa* L., by 30 to 40%, but CO₂ enrichment of 2,500 to 20,000 μmol · mol⁻¹ reduced seed yield 25 to 35% although it did not affect vegetative growth. Additionally, elevated CO₂ levels of 1,000 to 10,000 μmol · mol⁻¹ increased ethylene synthesis in some plants (Bugbee et al. 1994). Even at 10,000 μmol · mol⁻¹ CO₂ however, plants were never completely sterile.

Of the possible causes of our wheat sterility, only two seemed to have a high probability of actually being causal: microgravity and the presence of ethylene in the Mir atmosphere. Ethylene has long been known to cause male sterility and other effects in cereals (e.g., Abeles et al. 1992, Bennett and Hughes 1972, Keyes and Sorrells 1990, Rowell and Miller 1971, Voeselek et al. 1997). In one study, ethylene generated from ethephon (2-chloroethylphosphonic acid) spray applications induced abortion of sporogenous cells at both pre- and postmeiotic stages of microsporogenesis in barley (Colhoun and Steer 1983).

It is known that plants produce ethylene in response to abiotic and biotic stresses throughout their development and that ethylene levels even below 1 μmol · mol⁻¹ (equivalent to ppm by volume: ethylene · air⁻¹) will induce adverse biological activity (Abeles et al. 1992, Voeselek et al. 1997). Moreover, wheat plants have been shown to produce ethylene during vegetative growth (Petruzzelli et al. 1994) and grain filling (Beltrano et al. 1994, Labrana et al. 1991). Finally, various species of fungi are known to produce ethylene (see ref-

erences in Abeles et al. 1992), and over 100 species of fungi were identified in the Mir Space Station (L. Chernova, IMBP, Moscow, Russia, personal communication, 1997). A white fungus grew at the base of our Super Dwarf wheat plants although it did not appear to be directly harmful.

Although the Mir atmosphere had not previously been analyzed for ethylene, thirteen gas samples had been collected during our 1996 experiment, and when these were re-analyzed they were found to contain from 1.1 to 1.7 $\mu\text{mol} \cdot \text{mol}^{-1}$ ethylene with levels of about 1.4 $\mu\text{mol} \cdot \text{mol}^{-1}$ ethylene consistently during the anthesis period (Bingham et al. 1996a, James et al. 1998). Thus, we established the hypothesis that the observed sterility was caused by ethylene and not by microgravity. Three ways to test this hypothesis were apparent:

1. Add ethylene scrubbers to the equipment used in Mir to grow wheat, and then repeat the experiment. This is certainly the most obvious test, but neither NASA nor the Russian Space Agency would support such an experiment. (It was said to be too costly to develop the necessary equipment at that time.) Such an experiment will surely be carried out in future space studies.
2. Use a wheat cultivar that is more resistant to ethylene in the existing equipment. This has been done successfully with Apogee, a cultivar developed specifically for space studies and thus short enough to grow in Svet on Mir. Recently, Klassen and Bugbee (2000) have shown that Apogee is less sensitive to ethylene than the Super Dwarf wheat cultivar. All Super Dwarf plants receiving 1 $\mu\text{mol} \cdot \text{mol}^{-1}$ ethylene or higher were 100 % sterile, while there was some Apogee seed set at that level of ethylene. Probably because of the ethylene in Mir, only a few seeds of Apogee were produced, but these were carried through a second generation; that is, seed-to-seed-to-seed (Levinskikh et al. 1999a). Details will be reported elsewhere.
3. See if Super Dwarf wheat, grown under ethylene concentrations comparable to those in the Mir atmosphere during our 1996 experiment, exhibits identical symptoms to those observed in our Mir wheat, which in addition to the sterility included shortened leaves and stems, excessive tillering, and various microscopic features of the wheat heads. This is the approach described in this paper.

In spite of the known effects of ethylene on cereals, it was necessary to carry out these experiments because, of the studies on cereal sterility known to us (e.g., Bennett and Hughes 1972, Foster et al. 1991, Keyes and Sorrells 1990, Rowell and Miller 1971, and Taylor et al. 1991), all but one used ethephon (2-chloroethylphosphonic acid; Ethrel) in solution applied to their test plants as the source of ethylene, making it impossible to compare their results with our own. The one exception (Reid and Watson 1985) grew oats (*Avena sativa* L.) and not wheat; ethylene reduced the number of florets/plant from 464 in the zero ethylene control to only two in the 0.150 $\mu\text{mol} \cdot \text{mol}^{-1}$ ethylene treatment. To our knowledge, the results reported

here are the first to test a series of low ethylene concentrations on growth and reproduction of wheat, especially at high CO_2 concentrations.

Although our 1996/97 experiments in Mir were the first to produce quantities of plant biomass in microgravity comparable to those produced in ground controls, there is a relatively long history of plant experiments in space. Scientists in the former Soviet Union were able to grow plants for several months on Salyut-4, 6 and 7 (Dubinin et al. 1977, Gorkin et al. 1980, Kodyum et al. 1983, Merkys et al. 1981, Merkys and Laurinavichyus 1983) and in their orbital space station Mir (Mashinsky et al. 1994, Nechitailo and Mashinsky 1993). Working with carrot (*Daucus carota* L.), cucumber (*Cucumis sativus* L.), dill (*Anethum graveolens* L.), onion (*Allium cepa* L.), pea (*Pisum sativum* L.), radish (*Raphanus sativus* L.), and wheat (*Triticum aestivum* L.) onboard various spaceflight vehicles, the Soviets obtained fair vegetative growth from germinated seeds, but plant death occurred routinely at or before the flowering stage. Moreover, numerous long-term plant experiments in space failed to produce normal growth (vegetative biomass or seed yield) compared with those grown on earth (Dutcher et al. 1994, Halstead and Dutcher 1987, Mashinsky et al. 1988, Merkys and Laurinavichyus 1983, Nechitailo and Mashinsky 1993).

After many attempts, however, Merkys and Laurinavichyus (1983) were successful in growing *Arabidopsis thaliana* (L.) Heynh. from seed-to-seed in Salyut-7. Yet the seeds produced in space were shrunken and exhibited a 38 % reduction in germination compared to seeds from earth-grown control plants. Cowles et al. (1984) also reported reduced vigor of oats (*Avena sativa* L.), mung bean (*Vigna radiata* (L.) Wilczek.), and pine (*Pinus elliotti* Engelm.) seedlings on the shuttle Orbiter STS-3. Conger et al. (1998) also observed that 11 days on shuttle Orbiter STS-64 dramatically suppressed the initiation and development of somatic embryos from mesophyll cells of orchard grass (*Dactylis glomerata* L.) grown *in vitro*, whereas normal somatic embryos were initiated and developed from similarly cultured leaf segments in earth-grown controls. Krikorian and O'Connor (1984) observed chromosome breakage and bridge formation in space-grown oat and sunflower (*Helianthus annuus* L.) seedlings.

More recently, the reports of Kuang et al. (1996) are encouraging as they observed normal development of seeds in *Arabidopsis thaliana* plants that flowered during 11 days in space on shuttle Orbiter STS-68. Also, Krikorian et al. (1981) flew totipotent cells and cell clusters of carrot onboard Cosmos 782 and 1129 Biosatellites. They observed that carrot embryos developed into well-defined plants with no detectable differences between space- and earth-grown plantlets.

Mashinsky et al. (1994) achieved the formation of a single spike on Super Dwarf wheat grown in a small cylinder (called Svetoblock-M) onboard the Mir, but it remained seedless. Upon return to earth, tillers that formed while onboard Mir matured and produced seed when placed under higher light intensity and ambient CO_2 . The experimenters did not as-

cribe these poor responses to microgravity, but to environmental and micro-environmental factors on Mir: low photosynthetic photon flux, short photoperiods, high to toxic levels of CO₂ (typical for virtually all space experiments), inadequate water distribution in the rooting medium, or perhaps some unknown factor(s) that delayed the flowering process. Mashinsky et al. (1994) suggested that wheat was capable of forming reproductive organs in spaceflight provided conditions similar to those required for plant growth on earth were met.

To reiterate, the objectives of our earth-grown Super Dwarf wheat experiments were to establish a dose-response curve of Super Dwarf wheat to low levels of ethylene gas in the presence of elevated CO₂, observing vegetative growth and floral development. We present here the comparative floret development of Mir-grown and ethylene-treated, earth-grown wheat plants with analyses of the cause(s) of Super Dwarf wheat sterility.

Materials and Methods

Flight experiment: Super Dwarf wheat plants growing conditions on Mir

The Svet (means light in Russian) growth chamber onboard the Russian space station Mir (means peace in Russian) has 0.1 m² of growing area, and accommodates plants up to 40 cm tall (Bingham et al. 1995, Salisbury 1997). The Svet was designed by Russian (Institute of BioMedical Problems: IMBP) and Bulgarian scientists and built at the Space Research Institute, Sofia, Bulgaria under the supervision of Tania Ivanova. Ground-based controls at IMBP in Moscow were grown in a Svet unit identical to the one on Mir. Fluorescent lamps (Osram Delux S, 11W, Sylvania cool white, Branch of Siemens Co., Danvers, MA) provided light at a photosynthetic photon flux (PPF = micromoles of photons between 400 and 700 nm per square meter second) of 400 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with 18 to 23 h of light and 1 to 6 h darkness and temperature regimes of ca. 27/21 °C (day/night). An environmental control system capable of measuring several parameters including CO₂ was built at Utah State University (Space Dynamics Laboratory) and installed in Svet. Detailed description of the hardware can be found in Bingham et al. (1995, 1996 a, b).

The root module contained two compartments, each 31.9 cm long, 17.5 cm wide, and 9.0 cm deep (Salisbury 1997). These were filled with a plant-nutrient-loaded clinoptilolite (Cp) zeolite (1–2 mm diameter particles) called Balkanine (supplied by Tania Ivanova). The zeolite is a natural hydrated aluminosilicate mineral with a high degree of internal tunneling and cation exchange capacity (Boettinger and Graham 1995, Ming and Mumpton 1989). Twenty-six seeds of wheat (*Triticum aestivum* L. cv. Super Dwarf: CIMMYT selection CMH79.481-1Y8B-2Y-2B-OY; Salisbury et al. 1998), previously attached to plastic strips with a water-soluble white glue (Levinskikh et al. 1999 a, b) were placed 1 cm below the surface between two cotton-fabric wicks in each of the two rows (26 seeds/row) of each root compartment for a total of 104 seeds. Water was injected through hydroaccumulators embedded in the Balkanine medium in each of the two compartments and transferred by capillary action through the wicks directly to the seeds and the surrounding Balkanine. Water quantity and distribution

were measured hourly by 16 moisture sensors (two rows of four each in each compartment, placed at 4.0 and 7.0 cm in the substrate). Approximately 65 % relative water content was maintained in each of the root compartments (Bingham et al. 1996 a, b, Salisbury 1997, Yendler et al. 1996).

Flight experiment: harvest, preparation, and examination of plant material from Mir

Wheat plants, both ground controls and on Mir, were sampled at 21, 39, 52, and 63 d and harvested at 123 d from emergence. Fresh samples were stored in plastic bags containing aqueous fixative (40 g · L⁻¹ formaldehyde, 10 g · L⁻¹ glutaraldehyde, buffered with 0.1 mol · L⁻¹ cacodylate at pH 7.2; McDowell and Trump 1976). Dry samples were stored in «Sorb-it-Silica™» (Silica Gel, Fisher Scientific, Pittsburgh, PA). Upon return to earth, all Mir- and earth-grown spikes were examined over a narrow slit of light (makes seeds visible) to monitor for seeds. Mir-grown spikes showed 100 % sterility compared with relatively good seed set on earth-grown spikes (Levinskikh et al. 2000).

Dose-response experiment: plant growing conditions on earth, ethylene treatment

Super Dwarf wheat seeds were planted into 0.071 m² hydroponic plastic flats and covered with 2 mm diameter extruded diatomaceous earth (Isolite, Sundine Enterprises, Arvada, CO). Plastic flats were placed in six plexiglass hydroponic cylinders (30 cm dia., 50 cm tall, total volume = 35 L) in a controlled-environment chamber (Percival, Model PT-80, Boone, IA) at 23 ± 2 °C day/night. Air flow to each cylinder was maintained at 20 L · min⁻¹ and regulated by large rotameters (Model RMA, Dywer Instruments Inc., Michigan City, IN). Fans in the top of each cylinder mixed the internal air and maintained relative humidity at about 80 %. Oxygen in the nutrient medium, which remained constant at 85 % of saturation or greater, was replenished as it cascaded back into the reservoir. Atmospheric pressure at Logan, UT (1400 m elevation) was 86 ± 1 kPa (85 % of sea level). Carbon dioxide level was maintained at 1200 $\mu\text{mol} \cdot \text{mol}^{-1}$ (micromoles of CO₂ per mole of air) throughout the study (Grotenhuis and Bugbee 1997). Pure CO₂ and ethylene gases were mixed with filtered air pumped from outside the building, and plants' exposure to ethylene gas dilutions of 0, 1, 3, 10, or 20 $\mu\text{mol} \cdot \text{mol}^{-1}$ began 7 d after emergence. Recirculating nutrient solutions specifically developed for wheat were pumped into each cylinder from the reservoir by a magnetic-drive pump at a rate of 0.08 L · s⁻¹ (Grotenhuis and Bugbee 1997). Gas levels of each plant cylinder were controlled by small rotameters (Model RMA, Dywer Instruments Inc.) and monitored by gas chromatography.

Plant exposure to cool-white light (PPF between 32.4 and 36 $\text{mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, given at 450–500 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) began 10 d after seeding with a daily 12 h photoperiod. This day was appointed as the emergence date as it was the plants' first exposure to light. Plants were thinned to ca. 30 plants per cylinder at emergence. Fourteen days after planting, the daily photoperiod was increased to a 20/4 d/n regime. Mylar skirts were placed around the cylinders and maintained at the same height as the plants to minimize the influence of side lighting and to imitate canopy conditions noted in the center of field plots.

Table 1. Size of anthers and pollen grains in standard wheat varieties compared to Mir-grown and ethylene-treated, earth-grown Super Dwarf wheat.

	Anther			Pollen grain Dia. (μm)	References
	Ln (mm)	Width (mm)	Size (mm^2)		
Std. Wheat	3.19–4.41	0.57–1.07	1.82–4.72	47.1–52.7	Kherde et al. 1967 De Vries 1971
Chinese Sp.	3.45	–	–	–	
Super Dwarf					
Grnhsc	1.53 \pm 0.05	0.41 \pm 0.01	0.63 \pm 0.03	46.1 \pm 1.6	
Mir	1.38 \pm 0.13	0.33 \pm 0.02	0.46 \pm 0.03	41.8 \pm 1.4	
C ₂ H ₄					
NASA/ARC					
2 $\mu\text{mol} \cdot \text{mol}^{-1}$	–	–	–	33.8 \pm 0.6	
USU					
Control				43.0 \pm 0.05	
1 $\mu\text{mol} \cdot \text{mol}^{-1}$				38.4 \pm 0.08	
3 $\mu\text{mol} \cdot \text{mol}^{-1}$				37.9 \pm 0.09	
10 $\mu\text{mol} \cdot \text{mol}^{-1}$				36.1 \pm 0.08	
20 $\mu\text{mol} \cdot \text{mol}^{-1}$				35.6 \pm 0.25	

Dose-response experiment: harvest, preparation, and examination of ethylene-treated plants on earth

Plants were sampled at 63, 77, 85, and 88 d after emergence for morphological and SEM and LSCM studies of developmental stages of ontogeny. Morphological data measured included plant height (mm), number of tillers, number of leaves per tiller, spike length (mm), awn length (mm), internode length (mm), number of spikelets per spike, number of nodes per rachis, number of florets per spikelet, and percent seed set. The experiment concluded when the control plants reached physiological maturity at 88 days after emergence, determined by the loss of green color in the seeds.

Scanning electron microscopy (SEM) procedures

Wheat flowers consisting of pistils, stamens, and lodicules were excised from the fixed spikelets, dehydrated with graded series of ethyl alcohol, acetone, and tetramethylsilane (TMS), and evaporated overnight at 25 °C. The processed floral tissues from Mir and the ground controls were mounted onto circular aluminum stubs (15 mm dia) with an inert glue, «Torr Seal™» (Ted Pella, Inc., 3595 Mountain Lakes Blvd., Redding, CA). To enhance the contrast and density of the tissues, between 12.6 and 21.0 nm of gold (Au) and palladium (Pd) (60% : 40%) were evaporatively applied to the tissue surfaces with an Ion Beam Sputterer (Model IBS/TM200S, VCR Group, Inc.). The ion gun produced 5 kV and 2 mA. Specimens were examined and photographed with a scanning electron microscope (Hitachi S-4000) at an accelerating potential between 2 and 5 kV.

Laser scanning confocal microscopy (LSCM) procedures

Anthers were crushed to expose pollen grains and incubated in 250 μL propidium iodide in 50% ETOH and 100 μL of dimethyl sulfoxide (DMSO) for one hour in darkness in 2 mL microfuge tubes. After centrifugation for 5 min at 1200 rpm, the supernatant was decanted, and a portion of the pellet was placed in 100 μL of Prolong Antifade

Kit (Molecular Probes, Eugene, OR). Slides were cover-slipped and sealed with cyanoacrylate glue to minimize oxidation of the dye. The BioRad MRC-1024 LSCM was equipped with an Argon–Krypton (Ar–Kr) laser. Pollen grains were optically sectioned and examined with the LSCM interfaced with a Nikon Eclipse TE-300-inverted microscope. By raising or lowering the sample in discreet steps, thin optical fluorescent sections were generated and with the appropriate computer software were combined into a composite image. The Ar–Kr laser produces light centered at 488 and 568 nm, with the generated wavelengths overlapping at 536 nm. The light at 536 nm caused the propidium iodide in the pollen nuclei to fluoresce, generating light centered at 617 nm.

Results and Discussion

Flight experiment: observations on Mir-grown Super Dwarf wheat plants

Emergence percentages recorded from seeds in compartments one and two of the root module 7 d after planting were 56% and 73% on Mir and 75% and 90% on earth. Seedling growth was vigorous until the onset of anthesis, at which time the main spike and primary tiller spikes began to senesce, and new tillers with spikes were formed producing 5–8 tillers per plant on Mir compared with 3–5 on ground controls. Video tapes (14 total) were taken of plants on Mir and transmitted to earth at intervals. These videos showed what appeared to be normal ontological stages of development with the largest biomass of wheat plants (280 spikes harvested) ever produced in microgravity. Although we only received fixed or mature, dry plants, and almost half of the plants were removed for sampling, we estimate that the fresh mass would have exceeded a kilogram if all plants had been allowed to mature. Because of the repeated tillering, anthesis continued



Figure 1. SEM of floret harvested from a Super Dwarf inflorescence 63 d after emergence in Svet onboard Mir and stored in chemical fixative. The stigma (ST) has not yet unfurled. Stamens, pistil, and the enlarged lodicules (L) indicate that the floret is in the pre-anthesis stage. The Anther (A) appears normal. (Anther length: ca. 1.4 mm; see Table 1.)



Figure 2. Super Dwarf wheat floret harvested from earth-grown controls. Stamens, pistil, and enlarged lodicules indicate that this floret was at the onset of anthesis. The feathery stigma of the ovary has unfurled. Anthers have started to dehisce, but pollen grains were not yet evident on the stigmatic surfaces. (Anther length: ca. 1.5 mm; see Table 1.)



Figure 3. Scanning electron micrograph of stamens and pistil excised from a wheat plant grown in Svet onboard the Mir Space Station. Note the dry, withered and shrunken conditions of the Stamens, pistil and lodicules.

for approximately 40 d. Plants were all sterile upon harvest and return to earth. No pollen grains were evident on the stigmatic surfaces of Mir ovules in contrast to the commonly observed pollen-covered stigma of developing ground plants. Measurements of wheat anthers from SEM micrographs indicated that anthers developed on ground-control plants were 10 % longer and 20 % wider than those from Mir (Table 1). Also, pollen grain diameters were 9 % greater in controls than those from Mir-grown plants.

In Mir-grown plants, development of the primary and tiller stems produced normal-looking spikes over a six-week period. Examination of Mir-grown florets (Fig. 1) containing pistils, stamens, and lodicules indicated that, although they were delayed in development, they were developing similarly to those produced in ground-based growth chambers (Fig. 2). The swollen lodicules and the firm pistil and stamens suggests that the florets were approaching or were at the onset of anthesis. All florets from Mir that were examined, however, indicated a cessation of development in about the same stage of ontogeny; that is, prior to anthesis, at which time the floret began to senesce, the anthers did not dehisce, and pollen grains began to collapse (Fig. 3). The pistil, stamens, and lodicules from a floret harvested 123 d after emergence clearly showed that they ceased development and began to senesce prior to anthesis; all of the organs appeared to have collapsed (Fig. 1–3). Manually or accidentally ruptured anthers were observed to contain an abundance of pollen grains (Figs. 4 and 5), and the exine surfaces of Mir-grown pollen grains were similar to those produced on the ground. Pollen grains from ground controls harvested at full maturity showed only minor shrunken grains (Fig. 5).



Figure 4. A manually ruptured anther showing senesced, collapsed pollen grains from Mir-grown wheat harvested at 63 d after emergence. (Pollen diameters: ca. $42\text{ }\mu\text{m}$; see Table 1.)



Figure 5. A ruptured anther showing senesced pollen grains from earth-grown wheat harvested 63 d after emergence. Note the number of pollen grains still in a firm, circular shape. (Pollen diameters: ca. $46\text{ }\mu\text{m}$; see Table 1.)

Dose-response experiment: observations on ethylene-treated, earth-grown Super Dwarf wheat plants

As noted in the introduction, it is well known that ethylene gas (nearly always supplied as ethephon) can cause male sterility in cereals, and measurements of samples of Mir atmosphere indicated the presence of ethylene at levels of 1.1 to $1.7\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$, consistently about $1.4\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$ during the anthesis period (Bingham et al. 1996 a, James et al. 1998). Thus we directed our efforts at studying responses of Super Dwarf wheat to various concentrations of gaseous ethylene. (Some members of our group, working with others, have now extended these studies to ethylene concentrations as low as $0.050\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$; the results will be published elsewhere.)

To confirm that the Super Dwarf wheat cultivar was sensitive to the level of ethylene measured onboard the Mir, we exposed plants to 0 , 1 , 3 , 10 , and $20\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$ of ethylene gas and $1200\text{ }\mu\text{mol}\cdot\text{mol}^{-1}\text{ CO}_2$. There were significant reduc-



Figure 6. SEM of an earth-grown wheat floret at anthesis. This floret had been exposed to $20\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$ ethylene for 81 d. The large bodies below the middle anther are lodicules, which have shrunk slightly. (Anther lengths: ca. 1.3 mm .)

Table 2. Morphometric responses of earth-grown Super Dwarf wheat plants at elevated CO_2 ($1200\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$) and varying levels of ethylene gas – Logan, UT.

Ethylene Level $\mu\text{mol}\cdot\text{mol}^{-1}$	Plant Height (mm)	No. of Tillers	Spike Ln (mm)	Awn Ln (mm)	No. Rachis Nodes	Florets/ Spikelet	Percent Fertility
0	391 ± 8.2	5.6 ± 1.3	57.9 ± 1.5	50.9 ± 2.0	17.2 ± 0.4	3.9 ± 0.1	53.0 ± 3.5
1	275 ± 7.3	8.0 ± 0.7	44.2 ± 1.7	8.8 ± 1.1	17.2 ± 1.1	10.1 ± 1.1	0.0
3	251 ± 4.7	12.6 ± 2.2	40.4 ± 1.9	6.7 ± 0.2	16.8 ± 0.5	12.5 ± 1.1	0.0
10	221 ± 13.4	15.4 ± 5.2	40.0 ± 3.2	6.6 ± 0.5	16.2 ± 0.4	11.1 ± 1.3	0.0
20	199 ± 5.5	15.2 ± 3.2	43.8 ± 0.6	9.3 ± 1.4	15.8 ± 1.0	11.5 ± 1.7	0.0

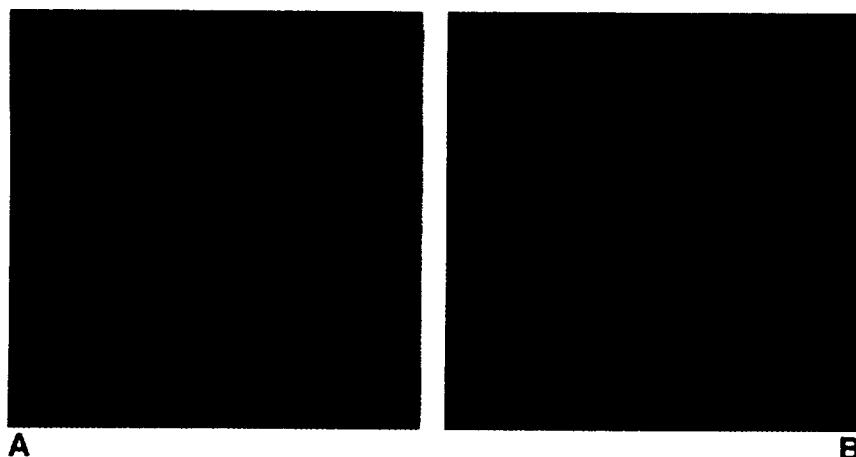


Figure 7. A. LSCM of pollen grains from control Super Dwarf wheat showing three propidium-iodide-stained nuclei. B. LSCM of pollen grains of Super Dwarf wheat exposed to $1 \mu\text{mol} \cdot \text{mol}^{-1}$ ethylene. Note that some pollen grains have only one or two nuclei.

tions in plant height, spike length, awn length, number of rachis nodes on the spike, and percent fertility, whereas number of tillers and number of florets per spikelet increased significantly with increased ethylene levels (Table 2). Spike and tiller awn lengths, number of rachis nodes, and number of florets per spikelet decreased with increased levels of ethylene (data not shown). Ethylene was consistent in inducing epinasty in leaves of primary and tiller plants (a length-wise curling into a cylinder). All the ethylene-treated plants in this experiment exhibited 100 % sterility. SEM examination of florets showed that their development proceeded normally but ceased prior to anthesis, and the anthers did not dehisce, thus mimicking Mir-grown wheat (Fig. 6). LSCM examination of pollen grains exposed to $1 \mu\text{mol} \cdot \text{mol}^{-1}$ ethylene or higher exhibited zero, one, and occasionally two, but rarely three nuclei, whereas those from earth-grown control plants were normally trinucleate (Fig. 7). At 10 and $20 \mu\text{mol} \cdot \text{mol}^{-1}$ ethylene, 90–95 % of the pollen grains did not contain any nuclei. An examination of 8 to $10 \mu\text{m}$ paraffin serial sections with a light microscope (Moscow State University) also indicated that pollen grains in the Mir-grown wheat had one or two, but rarely three nuclei as compared to the trinucleate stage observed in fertile earth-grown florets (Levinskikh et al. 1999 a, b, 2000, Veselova et al. 1999).

As noted, both anthers and pollen were smaller in ethylene-treated plants than in control plants (Table 1). Bennett and Hughes (1972) reported similar results although they used ethephon instead of gaseous ethylene. They noted that in florets in which male sterility had been induced, anther development was abnormal. Also, anthers from treated plants were smaller in size, and extrusion and dehiscence often failed compared with normal anthers. On rare occasions when dehiscence did occur, sterile pollen was released. Pollen grains did not contain any starch grains or elongated sperm nuclei, and sometimes more than three nuclei were observed. We have seldom observed three nuclei in pollen grains produced on the Mir or in ethylene-treated plants and never more than three.

The development of primary and numerous tiller spikes of wheat occurred in all of the ethylene treatments, but anthers that formed on the inflorescences did not dehisce nor did they have any fertile pollen. Flowering in wheat as in other seed-producing plants consists of a number of complex, sequential stages of ontogeny that involve pollen and embryo sac development, pollination, fertilization, and embryogenesis. Ethylene gas interrupts a crucial stage of reproductive development; namely, pollen development and anther dehiscence, thus causing a failure of seed set as reported earlier (Bennett and Hughes 1972, Reid and Watson 1985, Rowell and Miller 1971, Taylor et al. 1991).

Evidence supporting ethylene as the cause of wheat sterility onboard Mir

Ethylene is a known potent inhibitor of seed set in wheat and other cereals by inducing male sterility. Ethylene also promotes tillering, reduces stem growth, and causes leaf epinasty. We have observed all these characteristics in Mir-grown wheat.

Although the cellular processes that regulate anther-cell differentiation and pollen release are not known, the sequential events leading to viable pollen begin with meiosis and proceed in a precise chronological manner (Goldberg et al. 1993). There are, however, many sites along the developmental pathway in which disruptions may occur. The presence of pollen that appeared to be normal suggests that the initial phases of microsporogenesis occurred in the wheat plants grown onboard the Mir Space Station, but the anthers did not dehisce. Expansion of the ovary in Mir-grown wheat florets suggests that megasporogenesis was initiated as well as the swelling of the lodicules preparatory to anthesis (Leighty and Sando 1924).

Based on Super Dwarf wheat responses in this study, levels of ethylene and CO_2 onboard Mir were critical during the plants' development. Thus, future flight experiments with crop plants must consider scrubbers to lower or remove ethylene, which interferes with normal anther development and dehiscence.

cence and causes abnormal nucleation of pollen grains leading to male sterility. CO₂ levels might also have to be reduced. Installation of filters to remove impurities from the atmosphere were thought to have contributed to the success of growing *A. thaliana* through an entire life cycle on Salyut-7 (Merkys and Laurinavichyus 1983).

Considering the vigorous growth of our plants, we have every reason to expect that – in spite of the known sensitivity of plants to gravity – wheat and other plants can produce near-normal seed yields in microgravity when all other environmental factors are maintained at appropriate levels (Levinskikh et al. 1999 a, b, 2000). As noted in the Introduction, these expectations were at least partially confirmed by the few seeds produced with Apogee wheat grown in Mir, even with its atmospheric ethylene (Levinskikh et al. 1999 a).

Acknowledgements. The authors thank Tania Ivanova and coworkers, Space Research Institute, Sofia, Bulgaria, for construction and maintenance of the growth chamber, Svet. Appreciation is expressed to Tad Savage, Kristina Lagel, David Pletcher, Sally Greenawalt, and Terry Schnepf at the NASA Ames Research Center for administering and providing technical support; to Linda Gillespie and Mary Ann Clark, Dr. Salisbury's technician and his secretary, for their able assistance. Finally, we thank the Department of Biology, USU, for use of the scanning electron microscope and the laser scanning confocal microscope.

This research was supported by NASA Grant NCC 2-831 and the Utah Agricultural Experiment Station, Utah State University, Logan, UT 84322-4810. Approved as journal paper Number 6057.

References

- Abeles FB, Morgan PW, Saltveit ME Jr (1992) Ethylene in Plant Biology. Academic Press, San Diego, CA
- Beltrano J, Carbone A, Montaldi E, Guimmet JJ (1994) Ethylene as promoter of wheat grain maturation and ear senescence. *Plant Growth Regul* 15: 107–112
- Bennett MD, Hughes WG (1972) Additional mitosis in wheat pollen induced by Ethrel. *Nature* 240: 566–568
- Bingham GE, Salisbury FB, Campbell WF, Carman JG, Bubenheim DL, Yendler B, Sytchev VN, Berkovitch YuA, Levinskikh MA, Podolsky I (1995) The Space Lab-Mir-1 «Greenhouse-2» experiment. *Adv Space Res* 18: 225–232
- Bingham GE, Brown SB, Salisbury FB, Campbell WF, Carman JG, Jahns G, Bubenheim DL, Pletcher D, Yendler B, Sytchev VN, Levinskikh MA, Podolsky I, Ivanova I, Kosgtov P, Sapunovca S (1996 a) Environmental measurements observed during the Greenhouse-2 experiment on the Mir orbital station. Committee on Space Research (COSPAR) meetings in Birmingham, England, 14–21 July 1996, Abstracts 31: 364
- Bingham GE, Brown SB, Salisbury FB, Campbell WF, Carman JG, Jahns G, Pletcher D, Bubenheim DB, Yendler B, Sytchev VN, Levinskikh MA, Podolski I (1996 b) Plant growth and plant environmental monitoring equipment on the Mir space station: Experience and data from the Greenhouse II Experiment. SAE Technical Paper Series No. 961364, 26th International Conference on Environmental Systems, Monterey, CA, July 8–11, 1996
- Bingham GE, Jones SB, Or D, Podolski I, Levinskikh MA, Sytchev VN, Ivanova T, Kostov P, Sapunova S, Dandolov I, Bubenheim DB, Jahns G, Pletcher D (1997) Microgravity effects on water supply and substrate properties in porous matrix root support systems. 48th International Astronautical Congress, Turin, Italy, October 6–10, Reprint IAF/IAA-97-G.3.03
- Boettinger JL, Graham RC (1995) Zeolite occurrence in soil environments: An updated review. In: Ming DW, Mumpton FA (eds) *Natural Zeolites '93: Occurrence, Properties, Use, Intl. Comm. on Natural Zeolites*, Brockport, NY, pp 23–37
- Bugbee BG, Salisbury FB (1988) Exploring the limits of crop productivity. I. Photosynthetic efficiency of wheat in high irradiance environments. *Plant Physiol* 88: 869–878
- Bugbee BG, Salisbury FB (1989) Current and potential productivity of wheat for a controlled environment life support system. *Adv Space Res* 9(8): 5–15
- Bugbee BG, Spanarkel B, Johnson S, Monje O, Koerner G (1994) CO₂ crop growth enhancement and toxicity in wheat and rice. *Adv Space Res* 14: 257–267
- Colhoun CW, Steer MW (1983) The cytological effects of the gametocides ethrel and RH-531 on microsporogenesis in barley (*Hordeum vulgare* L.). *Plant Cell Environ* 6: 21–29
- Conger BV, Tomaszewski Z Jr, McDaniel JK, Vasilenko A (1998) Space-flight reduces somatic embryogenesis in orchardgrass (*Poaceae*). *Plant Cell Environ* 21: 1197–1203
- Cowles JR, Scheld HW, Lemay R, Peterson C (1984) Growth and lignification in seedlings exposed to eight days of microgravity. *Ann Bot* 54: 33–48
- De Vries APH (1971) Flowering biology of wheat, particularly in view of hybrid seed production – a review. *Euphytica* 20: 152–170
- Dubinin NP, Glembotsky YaL, Vaulina EN, Merkis AI, Laurinavichius RS, Palmbakh LR, Grozdova TYa, Holikova TA, Yaroshyus AV, Mashinsky AL, Izupak EA, Konshin NI (1977) Biological experiments on the orbital station Salyut 4. *Life Sci Space Res* 15: 267–272
- Dutcher FR, Hess E, Halstead TW (1994) Progress in plant research in space. *Adv Space Res* 14: 159–171
- Foster KR, Reid DM, Taylor JS (1991) Tillering and yield responses to ethephon in three barley cultivars. *Crop Sci* 31: 130–134
- Goldberg RB, Beals TP, Sanders PM (1993) Anther development: Basic principles and practical applications. *Plant Cell* 5: 1217–1229
- Gorkin Yu, Mashinsky A, Yazdovsky V (1980) Birthday flowers: «Salyut-6», our commentary. *Pravda*, October 27, p 3 (In Russian)
- Grotenhuis T, Bugbee BG (1997) Super-optimal CO₂ reduces seed yield but not vegetative growth in wheat. *Crop Sci* 37: 524–532
- Halstead TW, Dutcher FR (1987) Plants in space. *Annu Rev Plant Physiol* 38: 317–345
- James JT, Limero TF, Beck SW, Yang L, Martin MP, Matney ML, Covington PA, Boyd JF (1998) Toxicological assessment of air contaminants. Shuttle-Mir Science Program Phase 1A. Research Postflight Science Report 4: 111–124
- Jiang L, Salisbury FB, Campbell WF, Carman JG, Nan R (1998) Studies on flower initiation of Super-Dwarf wheat under stress conditions simulating those on the Space Station, Mir. *J Plant Physiol* 152: 323–327
- Keyes G, Sorrells ME (1990) Mutations blocking sensitivity to gibberellic acid promote ethylene-induced male sterility in wheat. *Euphytica* 48: 129–139

- Kherde MK, Atkins IM, Merkle OG, Porter KB (1967) Cross pollination studies with male sterile wheats of three cytoplasm, seed size on F_1 plants, and seed and anther size of 45 pollinators. *Crop Sci* 7: 389–394
- Klassen SP, Bugbee BG (2000) Ethylene sensitivity of crops in controlled environments. *Amer Soc of Agron, Abstracts of Annual Meeting*, Madison, WI, U.S.A., p 126
- Kodyum EL, Sytnik KM, Chernyaeva II (1983) Peculiarities of genital organ formation in *Arabidopsis thaliana* (L.) Heynh. under space-flight conditions. *Adv Space Res* 3: 247–250
- Krikorian AD, O'Connor SA (1984) Karyological observations. *Ann Bot* 54: 49–63
- Krikorian AD, Dutcher FR, Quinn CE, Steward FC (1981) Growth and development of cultured carrot cells and embryos under space-flight conditions. *Adv Space Res* 1(14): 117–127
- Kuang A, Musgrave ME, Tucker SC (1993) Pollen development in *Arabidopsis thaliana* in microgravity. *Amer Soc Gravitational Space Biol Bulletin* 7: 62
- Kuang A, Musgrave ME, Matthews SW, Cummins DB, Tucker SC (1995) Pollen and ovule development in *Arabidopsis thaliana* under spaceflight conditions. *Amer J Bot* 82: 585–595
- Kuang A, Xiao Y, Musgrave ME (1996) Cytochemical localization of reserves during seed development in *Arabidopsis thaliana* under spaceflight conditions. *Ann Bot* 78: 343–351
- Labrana X, Vendrell M, Araus J (1991) Ethylene production of wheat flag leaves and ears during grain filling. *Plant Physiol Biochem* 29: 349–354
- Leighty CE, Sando WJ (1924) The blooming of wheat flowers. *J Agric Res* 27: 231–244
- Levinskikh MA, Sytchev VN, Podolsky IG, Bingham GE (1999 a) Final plant experiments on Mir provide second generation wheat and seeds. *Amer Soc Gravitational Space Biol Ann Meeting*, 4–7 Nov., Seattle, WA
- Levinskikh MA, Sychev VN, Derendyaeva TA, Signalova OB, Salisbury FB, Campbell WF, Bubenheim D (1999 b) Effects of some space flight factors on growth and development of Super Dwarf wheat cultivated in greenhouse. *Svet. Aviakosmicheskaya i Ekologicheskaya Meditsina (Russia)* 33(2): 37–41
- Levinskikh MA, Sychev VN, Derendyaeva TA, Signalova OB, Salisbury FB, Campbell WF, Bingham GE, Bubenheim DL, Jahns G (2000) Analysis of the spaceflight effects on growth and development of Super Dwarf wheat grown on the Space Station Mir. *J Plant Physiol* 156: 522–529
- Mashinsky AL, Nechitailo GS, Vaulina EN (1988) Space biology. *Biology (Moscow)* 10: 64
- Mashinsky AL, Ivanova I, Derendyaeva T, Nechitailo GS, Salisbury FB (1994) From seed-to-seed experiment with wheat plants under space-flight conditions. *Adv Space Res* 14(11): 13–19
- McDowell EM, Trump BF (1976) Histologic fixatives suitable for diagnostic light and electron microscopy. *Arch Pathol Lab Med* 100: 405–419
- Merkys AJ, Laurinavichyus RS (1983) Complete cycle of individual development of *Arabidopsis thaliana* (L.) Heynh. plants on board the «Salyut-7» orbital station. *Dokl Akad Nauk SSSR* 271(2): 509–512. English translation NASA Technical Memorandum 77576
- Merkys AJ, Laurinavichyus OY, Rupainene OY, Shvegzhdene DV, Yaroshius AV (1981) Gravity as an obligatory factor in normal higher plant growth and development. *Adv Space Res* 1: 109–116
- Ming DW, Mumpton FA (1989) Zeolites in Soils. In: *Minerals in Soil Environments* (2nd ed), SSSA Book Series, No. 1. 677 South Segoe Road, Madison, WI 53711, USA, pp 873–911
- Murgia M, Tucker SC, Musgrave ME (1992) Viability of *Arabidopsis thaliana* pollen in low oxygen. *Amer Soc Gravitational Space Biol Bulletin* 6: 52
- Nechitailo GS, Mashinsky AL (1993) Space biology: Studies at orbital stations. *Mir Publishers*, Moscow
- Petruzzelli L, Harden F, Reiss J (1994) Patterns of C_2H_4 production during germination and seedling growth of pea and wheat as indicated by a laser-driven photoacoustic system. *Environ Exp Bot* 34: 55–61
- Quebedeaux B, Hardy RWF (1973) Oxygen as a new factor controlling reproductive growth. *Nature* 243: 477–479
- Reid DM, Watson K (1985) Ethylene as an air pollutant. In: Roberts JS, Tucker GA (eds) *Ethylene and Plant Development*. Butterworths, London
- Rowell PL, Miller DG (1971) Induction of male sterility in wheat with 2-chloroethylphosphonic acid (Ethrel). *Crop Sci* 11: 629–631
- Salisbury FB (1993) Gravitropism: Changing ideas. *Hort Rev* 15: 232–278
- Salisbury FB (1997) Growing Super Dwarf wheat in Space Station Mir. *Life-Support Biosphere Sci* 4: 155–166
- Salisbury FB (1999) Growing crops for space explorers on the Moon, Mars, or in space. *Adv Space Biol Med* 7: 131–162
- Salisbury FB, Gillespie LS, Campbell WF, Hole P (1998) Ground-based studies with Super-Dwarf wheat in preparation for space flight. *J Plant Physiol* 152: 315–322
- Taylor JS, Foster KR, Caldwell CD (1991) Ethephon effects on barley in Central Alberta. *Can J Plant Sci* 71: 983–995
- Thomson CJ, Greenway H (1991) Metabolic evidence for stelar anoxia in maize roots exposed to low O_2 concentrations. *Plant Physiol* 96: 1294–1301
- Veselova TD, Ilyina GM, Dzhaliolova HH, Levinskikh MA, Sychev VN, Salisbury FB, Campbell WF (1999) The cytoembryologic investigation of Super Dwarf wheat grown on board the Mir orbital complex. *Aviakosmicheskaya i Ekologicheskaya Meditsina (Russia)* 33(2): 30–37
- Voesenek LACJ, Vriezen WH, Smekens MJE, Huitink FHM, Bogemann GM, Blom CWPM (1997) Ethylene sensitivity and response sensor expression in petioles of *Rumex* species at low O_2 and high CO_2 concentrations. *Plant Physiol* 114: 1501–1509
- Yendler BS, Webbon B, Podolsky I, Bula RJ (1996) Capillary movement of liquid in granular beds in microgravity. *Adv Space Res* 18: 233–237